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# Transportation of Poorly Soluble Drug Molecules from the Organic Phase to the Aqueous Phase by Using Phosphorylated Calixarenes

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**ABSTRACT:** This study is the first report on the extraction of poorly soluble drug molecules such as nifedipine, niclosamide, and furosemide from the organic phase to the aqueous phase by water-soluble *p*-phosphonate calix[*n*]arene receptors via a liquid−liquid phase extraction process. These water-soluble calixarene derivatives were easily obtained from the reaction between their corresponding chloromethylated derivatives and trimethyl phosphite. From the liquid−liquid phase extraction studies, it was observed that the size of the *p*-phosphonate calix[*n*]arenes changed the extraction percentage of selected drug molecules.

## INTRODUCTION

Niclosamide 5-chloro-*N*-2-chloro-4-nitrophenyl-2-hydroxybenzamide, nifedipine 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, and furosemide 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl) amino] benzoic acid are poorly water-soluble drug molecules and used as anthelmintics, calcium channel blockers, and loop diuretics, respectively.[1,2] The main problem of these molecules is poor aqueous solubility. A commonly used technique to increase the solubility of poorly water-soluble drugs is by supramolecular complexation.[3−5] Among the macromolecules used to solubilize drugs the cyclodextrins are the most widely used.[6] Cyclodextrins are a family of three major well-known cyclic oligosaccharides. The negligible cytotoxic effects of cyclodextrins are an important attribute in the application of drug carriers.[7] Along with the cyclodextrins and crown ethers, calixarenes are the third major class of supramolecular systems. The importance of calixarenes has been entirely recognized since pioneering studies of Gutsche.[8,9] Calixarenes are cyclic oligomers made of several phenolic units bounded with methylene bridges, which can adopt various conformations and form hydrophobic cavities. Calixarenes can be decorated with a wide variety of functional groups on the aromatic rings and/or the O-centers of the phenolic groups, the so-called upper (or wide) and lower (or narrow) rims of the calixarenes, respectively.[10,11] Calixarenes are sparingly soluble in aqueous media, and this property is the major problem for the calixarene use in biopharmaceutical applications. To overcome these limitations water-soluble groups containing positive or negative charges such as amine,[12] phosphonate,[13] and sulfonate[14] groups or neutral groups such as sulfonamide,[15] sugar,[16] and polyoxyethylene[17] but highly hydrophilic groups can be located on the lower or upper rim of calixarene skeleton. Calixarenes may selectively include various guests according to their size and hydrophobicity in a manner similar to cyclodextrins.[18−20] Although the Foor and Drug Administration (FDA) has currently not approved the use of calixarenes in medicines to date, the calixarenes have showed neither toxicity nor immune response.[20] This situation increases interest in their use in the biopharmaceutical applications beyond their current use for the chiral separation of molecules[21] and as complex forming agents to remove molecules from the environment.[22−24] To date, although several works about the effect of the water-soluble *p*-sulfonic calix[*n*]arenes on the solubility of drugs have been reported,[25−27] there is no other published extraction study between poorly soluble drug molecules and water-soluble *p*-phosphonate calix[*n*]arene receptors. The molecular size of the 4-sulfonic calix[*n*]arenes and the concentration of the calix[*n*]arenes significantly influenced the increase in the solubility of drug molecules such as nifedipine, niclosamide, and furosemide. The inclusion behaviors of water-soluble *p*-phosphonate calix[*n*]arene receptors toward nifedipine, niclosamide, and furosemide have not been explored so far by means of liquid−liquid phase extraction. Therefore, the aim of the present study was to explore the effect of water-soluble *p*-phosphonate calix[*n*]arene on the extraction behavior of nifedipine, niclosamide, and furosemide.

## MATERIALS AND METHODS

**Materials.** All starting materials and reagents used were of standard analytical grade from Alfa Aesar, Merck, and Aldrich, and some of them were used without further purification. Analytical thin layer chromatography (TLC) was performed using Merck prepared plates (silica gel 60 $F_{254}$). All reactions, unless otherwise noted, were conducted under a nitrogen atmosphere. Toluene was distilled from $CaH_2$. Methanol was distilled over CaO and stored over molecular sieves (Table 1). Anions were used as their sodium salts. The drying agent used was anhydrous $MgSO_4$. All aqueous solutions were prepared with deionized water that had been passed through a Millipore milli-Q Plus water purification system.

**Apparatus.** $^1H$, $^{13}C$, and $^{31}P$ NMR spectra were recorded on a Varian 400 MHz spectrometer in $CDCl_3$ or $D_2O$. Melting points were determined on an Electrothermal 9100 apparatus in a sealed capillary and are uncorrected. IR spectra were obtained on a Perkin-Elmer spectrum 100 FTIR spectrometer (ATR). UV−vis spectra were obtained on a Shimadzu 160A UV−vis spectrophotometer. Elemental analyses were performed using a Leco

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**Table 1. Characteristics of Reagents**

| chemical name | source | initial mole fraction purity | purification method | final mole fraction purity | analysis method |
|---|---|---|---|---|---|
| toluene | Sigma-Aldrich | 0.997 | distillation | 0.998 | GC[a] |
| methanol | Sigma-Aldrich | 0.997 | distillation | 0.998 | GC[a] |
| trimethylphosphite | Alfa Aesar | 0.970 | distillation | 0.984 | GC;[a] $^1$H NMR[b] |
| chloroform | Merck | 0.990−0.994 | - | - | - |
| diethyl ether | Merck | 0.997 | - | - | - |

[a] Gas chromatography. [b] Nuclear magnetic resonance spectroscopy.

**Table 2. NMR Spectra of the Receptors (4a−c, 5c, and 6c)**

| compounds | chemical shifts in $^1$H and $^{31}$P NMR spectra ($\delta$, ppm, 400 Hz, $D_2O$) |
|---|---|
| **4a** | $^1$H NMR: 2.61 (d, 8H, $-CH_2P$, $J_{HP}$ = 20.2 Hz), 3.72 (bs, 8H, $ArCH_2Ar$), 6.89 (s, 8H, ArH) |
| | $^{31}$P NMR: 20.17 |
| | $^1$H NMR[a]: 2.64 (d, 8H, $-CH_2P$, $J_{HP}$ = 18. Hz), 3.60 (bs, 8H, $ArCH_2Ar$), 6.80 (bs, 8H, ArH) |
| **4b** | $^1$H NMR: 1.24 (s, 18H, $-C(CH_3)_3$), 2.82 (d, 4H, $-CH_2P$, $J_{HP}$ = 19.2 Hz), 3.65 (bs, 4H, $ArCH_2Ar$), 4.20 (bs, 4H, $ArCH_2Ar$), 7.04−7.10 (m, 8H, ArH) |
| | $^{31}$P NMR: 20.04 |
| **4c** | $^1$H NMR: 1.21 (m, 27H, $-C(CH_3)_3$), 2.84 (d, 4H, $-CH_2P$, $J_{HP}$ = 19.8 Hz), 3.51−3.53 (bs, 4H, $ArCH_2Ar$), 4.25−4.28 (bs, 4H, $ArCH_2Ar$), 6.98−7.08 (m, 8H, ArH) |
| | $^{31}$P NMR: 21.30 |
| **5c** | $^1$H NMR: 2.69 (d, 12H, $-CH_2P$, $J_{HP}$ = 19.4 Hz), 3.19 (s, 18H, $ArOCH_3$), 3.87 (bs, 12H, $ArCH_2Ar$), 6.87 (s, 12H, ArH) |
| | $^{31}$P NMR: 19.85 |
| | $^1$H NMR[a]: 2.84 (d, 12H, $-CH_2P$, $J_{HP}$ = 19.6 Hz), 3.15 (s, 18H, $ArOCH_3$), 3.95 (s, 12H, $ArCH_2Ar$), 6.98 (s, 12H, ArH) |
| **6c** | $^1$H NMR: 2.83 (m, 16H, $-CH_2P$), 3.53 (s, 24H, $ArOCH_3$), 3.92 (bs, 16H, $ArCH_2Ar$), 6.93 (s, 16H, ArH) |
| | $^{31}$P NMR: 20.05 |
| | $^1$H NMR[a]: 2.90 (d, 16H, $-CH_2P$, $J_{HP}$ = 10.0 Hz), 3.50 (s, 24H, $ArOCH_3$), 4.00 (s, 16H, $ArCH_2Ar$), 7.00 (s, 16H, ArH) |

[a] Reference 13.

CHNS-932 analyzer. A Crison MicropH 2002 digital pH meter was used for the pH measurements.

**Synthesis.** Chloromethylated calix[*n*]arene derivatives 5,11,17,23-tetrachloromethyl-25,26,27,28-tetrahydroxycalix[4]arene **1a**, 5,17-bis(chloromethyl)-11,23-bis(*p-tert*-butyl)-25,26,27,28-tetrahydroxycalix[4]arene **1b**, 5,11,17-tris(*p-tert*-butyl)-23-chloromethyl25,26,27,28-tetrahydroxycalix[4]arene **1c**, 5,11,17,23,29,35-hexachloromethyl-37,38,39,40,41,42-hexamethoxycalix[6]arene **5a**, and 5,11,17,23,29,35,41,47-octachloromethyl-49,50,51,52,53,54,55,56-octamethoxycalix[8]arene **6a** and water-soluble sodium salts of *p*-phosphonato calix[*n*]arene 5,11,17,23-tetrakis(hydroxyphosphonoyl)methyl-25,26,27,28-tetrahydroxycalix[4]arene **4a**, 5,17-bis(hydroxyphosphonoyl)-methyl-11,23-di-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene **4b**, 5,11,17-tris(*p-tert*-butyl)-23-hydroxyphosphonomethyl-25,26,27,28-tetrahydroxycalix[4]arene **4c**, 5,11,17,23,29,35-hexakis(hydroxyphosphonoyl)methyl-37,38,39,40,41,42-hexamethoxycalix[6]arene **5c**, and 5,11,17,23,29,35,41,47-octakis(hydroxyphosphonoyl)methyl-49,50,51,52,53,54,55,56-octamethoxycalix[8]arene **6c** were prepared according to the modified literature method (Table 2),[13,28,29] while phosphorylated calix[*n*]arenes **2a**−**c**, **5b**, and **6b** as illustrated in Schemes 1 and 2 was prepared for the first time as follows.

**Synthesis of *p*-Phosphonato Calixarene Derivatives 2a−c, 5b, and 6b.** *General Procedure.* A sample of $4.8 \cdot 10^{-4}$ mol of corresponding chloromethylated calix[4]arene derivatives **1a**−**c**, **5a**, and **6a** in 5 $cm^3$ of chloroform was refluxed for 5 h with 5 $cm^3$ of trimethyl phosphite. An excess amount of unreacted trimethyl phosphite ($P(OCH_3)_3$) was then distilled under reduced pressure, and the obtained yellow oily residue was dissolved in a minimum amount of chloroform and then precipitated with an excess amount of diethyl ether. Obtained yellow precipitates were filtered off and washed with diethyl ether to give corresponding pure of *p*-phosphonato calix[4]arene derivatives **2a**−**c**, **5b**, and **6b**.

*5,11,17,23-Tetrakis(dimethoxyphosphonoyl)methyl-25,26,-27,28-tetrahydroxycalix[4]arene (**2a**).* Yield = (80 %, pale yellow). $^1$H NMR ($CDCl_3$): $\delta$ 10.03 (s, 4H, OH), 6.96 (s, 8H, ArH), 4.16−4.13 (bs, 4H, $ArCH_2Ar$), 3.65−3.62 (d, 12H, $J$ = 10.6 Hz, $POCH_3$), 3.52−3.50 (bs, 4H, $ArCH_2Ar$), 2.93−2.90 (d, 4H, $J$ = 21.2 Hz, $CH_2PO$). $^{13}$C NMR ($CDCl_3$): $\delta$ 147.11, 129.89, 128.93, 128.81, 53.21, 33.47, 31.63. $^{31}$P NMR ($CDCl_3$): $\delta$ 29.33. Anal. Calc.: $C_{40}H_{52}O_{16}P4$. C, 52.64; H, 5.74; P, 13.57 %. Found: C, 52.61; H, 5.72; P, 13.53 %.

*5,17-Bis(dimethoxyphosphonoyl)methyl-11,23-di-tert-butyl-25,26,27,28-tetrahydroxycalix[4]arene (**2b**).* Yield = (77 %, pale yellow). $^1$H NMR ($CDCl_3$): $\delta$ 10.18 (s, 4H, OH), 7.06 (s, 4H, ArH), 6.94 (s, 4H, ArH), 4.22−4.19 (bs, 4H, $ArCH_2Ar$), 3.65−3.62 (d, 12H, $J$ = 10.5 Hz, $POCH_3$), 3.56−3.52 (bs, 4H, $ArCH_2Ar$), 2.91−2.86 (d, 4H, $J$ = 21.2 Hz, $CH_2PO$), 1.23 (s, 18H, $C(CH_3)$). $^{13}$C NMR ($CDCl_3$): $\delta$ 149.91, 143.55 129.81, 128.88, 128.79, 53.43, 34.79, 33.57, 32.03, 31.61. $^{31}$P NMR ($CDCl_3$): $\delta$ 29.31. Anal. Calc.: $C_{42}H_{54}O_{10}P_2$. C, 64.61; H, 6.97; P, 7.93 %. Found: C, 64.57; H, 6.92; P, 7.91 %.

*5,11,17-Tris(p-tert-butyl)-23-dimethoxyphosphonomethyl-25,26,27,28-tetrahydroxycalix[4]arene (**2c**).* Yield = (83 %, pale yellow). $^1$H NMR ($CDCl_3$): $\delta$ 10.28 (s, 4H, OH), 7.08−6.98 (m, 8H, ArH), 4.26−4.23 (bs, 4H, $ArCH_2Ar$), 3.67−3.64 (d, 12H, $J$ = 10.8 Hz, $POCH_3$), 3.53−3.47 (bs, 4H, $ArCH_2Ar$), 2.96−2.91 (d, 4H, $J$ = 21.2 Hz, $CH_2PO$), 1.22 (m, 27H, $C(CH_3)$). $^{13}$C NMR ($CDCl_3$): $\delta$ 148.43, 146.86, 146.50, 144.77, 130.50,


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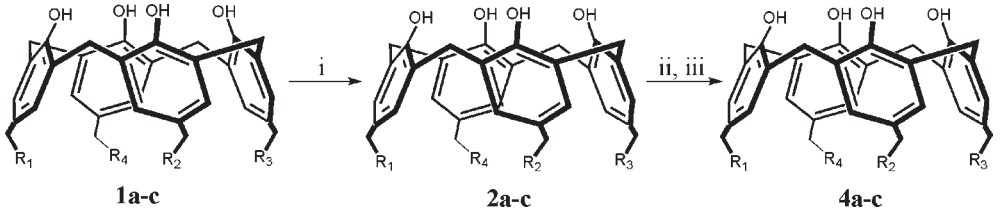
**Scheme 1. Synthetic Route for the Synthesis of Compounds 4a−c**[a]




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 1a: | Cl | Cl | Cl | Cl |
| 1b: | $(CH_3)_3$ | Cl | $(CH_3)_3$ | Cl |
| 1c: | $(CH_3)_3$ | $(CH_3)_3$ | $(CH_3)_3$ | Cl |
| 2a: | $PO(OCH_3)_2$ | $PO(OCH_3)_2$ | $PO(OCH_3)_2$ | $PO(OCH_3)_2$ |
| 2b: | $(CH_3)_3$ | $PO(OCH_3)_2$ | $(CH_3)_3$ | $PO(OCH_3)_2$ |
| 2c: | $(CH_3)_3$ | $(CH_3)_3$ | $(CH_3)_3$ | $PO(OCH_3)_2$ |
| 4a: | $POOHO^-Na^+$ | $POOHO^-Na^+$ | $POOHO^-Na^+$ | $POOHO^-Na^+$ |
| 4b: | $(CH_3)_3$ | $POOHO^-Na^+$ | $(CH_3)_3$ | $POOHO^-Na^+$ |
| 4c: | $(CH_3)_3$ | $(CH_3)_3$ | $(CH_3)_3$ | $POOHO^-Na^+$ |

[a] Reaction conditions: (i) trimethylphosphite, chloroform, reflux; (ii) BTMS and methanol, rt; (iii) 0.05 M NaOH.

129.08, 128.16, 127.85, 127.38, 126.34, 126.08, 125.90, 124.55, 53.17, 34.29, 34.20, 32.71, 32.40, 31.67. $^{31}$P NMR ($CDCl_3$): $\delta$ 29.34. Anal. Calc.: $C_{43}H_{55}O_7P$. C, 72.25; H, 7.75; P, 4.33 %. Found: C, 72.68; H, 7.82; P, 4.31 %.

*5,11,17,23,29,35-Hexakis(dimethoxyphosphonoyl)methyl-37,38,39,40,41,42-hexamethoxycalix[6]arene (**5b**).* Yield = (73 %, pale yellow). $^1$H NMR ($CDCl_3$): $\delta$ 6.81 (bs, 12H, ArH), 3.96−3.84 (m, 48H, $POCH_3$ and $ArCH_2Ar$), 3.69 (bs, 18H, $OCH_3$), 2.90−2.87 (m, 12H, $CH_2PO$). $^{13}$C NMR ($CDCl_3$): $\delta$ 151.53, 133.96, 128.65, 126.83, 61.03, 53.18, 33.41, 31.06. $^{31}$P NMR ($CDCl_3$): $\delta$ 29.11. Anal. Calc.: $C_{66}H_{90}O_{24}P_6$. C, 54.55; H, 6.24; P, 12.79 %. Found: C, 54.58; H, 6.32; P, 12.74 %.

*5,11,17,23,29,35,41,47-Octakis(dimethoxyphosphonoyl) methyl-49,50,51,52,53,54,55,56-octamethoxycalix[8]arene (**6b**).* Yield = (70 %, pale yellow). $^1$H NMR ($CDCl_3$): $\delta$ 6.90 (bs, 16H, ArH), 3.60−3.58 (m, 48H, $POCH_3$), 4.01 (s, 16H, $ArCH_2Ar$), 3.56 (s, 24H, $OCH_3$), 2.90−2.88 (m, 16H, $CH_2PO$),. $^{13}$C NMR ($CDCl_3$): $\delta$ 151.53, 133.96, 128.65, 126.83, 61.03, 53.18, 33.41, 31.06. $^{31}$P NMR ($CDCl_3$): $\delta$ 29.33. Anal. Calc.: $C_{88}H_{120}O_{32}P_8$. C, 54.55; H, 6.24; P, 12.79 %. Found: C, 54.46; H, 6.27; P, 12.69 %.

**Liquid−Liquid Extraction.** Into a vial was pipetted an aqueous solution (10 $cm^3$) containing a calixarene ligand (**4a−c**, **5c**, and **6c**) at a concentration of $4\cdot10^{-3}$ mol·$kg^{-1}$ and 10 $cm^3$ of $1.35\cdot10^{-4}$ mol·$kg^{-1}$ drug molecule in chloroform. The mixture was shaken vigorously in a stoppered glass tube with a mechanical shaker for 2 min and then magnetically stirred in a thermostatted water bath at 298 K for 5 h, respectively, and finally left standing for an additional 30 min. The concentration of drug molecule remaining in the organic phase was then determined spectrophotometrically at 347 nm for furosemide, 339 nm for niclosamide, and 319 nm for nifedipine. Blank experiments showed that drug extraction occurred in the absence of calixarene ligand (**4a−c**, **5c**, and **6c**). However, the percentage of the drug molecule in the absence of calixarene ligand (**4a−c**, **5c**, and **6c**) was observed around 4.6 %, 3.5 %, and 1 % for nifedipine, niclosamide, and furosemide, respectively.

The percent extraction ($E$ %) has been calculated as:

$$E\% = [(C_0 - C)/C_0]\cdot 100$$

where $C_0$ and $C$ are the initial and final concentrations of the drug molecule in organic phase before and after the extraction, respectively.

## RESULTS AND DISCUSSION

The syntheses of *p*-phosphonate calix[*n*]arenes **2a−c**, **5b**, and **6b** were performed by treatment of chloromethylated calix[*n*]arenes **1a−c**, **5a**, and **6a** which was synthesized according to modified literature procedure[13] with trimethyl phosphite as shown in Schemes 1 and 2. Trimethyl phosphite was chosen as a new phosphorylation reagent instead of triethyl phosphite owing to the easy elimination of unreacted phosphite compound from reaction conditions. For the purification of phosphoester derivatives **2a−c**, **5b**, and **6b**, neither chromatography nor lyophilization techniques from a cyclohexane suspension were used.[30] Phosphorylated calix[*n*]arenes **2a−c**, **5b**, and **6b** were easily converted their corresponding water-soluble *p*-phosphonate derivatives **4a−c**, **5c**, and **6c** by following a previously published literature procedure. All of the newly phosphorylated calix[*n*]arenes **2a−c**, **5b**, and **6b** were fully characterized by IR, $^1$H, $^{13}$C, and $^{31}$P NMR spectroscopy and elemental analysis. Spectroscopic data were in full agreement with those expected. The IR spectrum of compounds **2a−c**, **5b**, and **6b** was almost identical to that of corresponding chloromethylated calix[*n*]arenes **1a−c**, **5a**, and/or **6a** with small changes in wavenumbers. In the IR spectra of the *p*-phosphonate calix[*n*]arenes **2a−c**, **5b**, and **6b**, new peaks were seen around 1240 $cm^{-1}$ to 1245 $cm^{-1}$ attributable corresponding P═O bonds and 960 $cm^{-1}$ and 1055 $cm^{-1}$ attributable P−O−C bond stretching. The $^1$H NMR spectra of newly synthesized phosphoester derivatives **2a−c**, **5b**, and **6b** show two sets of doublets for the bridging methylene protons (Figure 1).

A typical AX pattern is observed for the methylene bridge $ArCH_2Ar$ protons around 3.56 ppm to 3.50 ppm ($J_{AB}$ = 13.1 Hz) and 4.26 ppm to 4.16 ppm for compounds **2a−c**, **5b**, and **6b** in the $^1$H NMR. The high field doublets around 3.56 ppm to 3.50 ppm for compounds **2a−c**, **5b**, and **6b** are assigned to the equatorial protons of methylene groups, whereas the low field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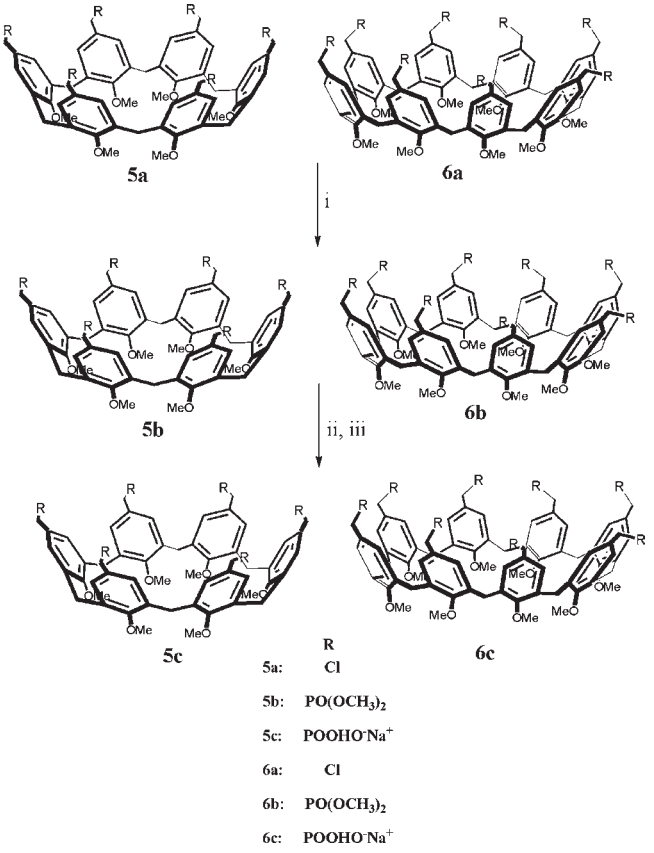
signals around 4.26 ppm to 4.16 ppm for compounds **2a**−**c**, **5b**, and **6b** are assigned to the axial protons in the $^{1}$H NMR. This NMR data demonstrated that these compounds are in the cone conformation. This situation is supported by $^{13}$C NMR data with Ar$CH_2$Ar resonance signals comprised between 31.00 ppm and 31.50 ppm. It is obvious that related compounds are symmetrical, and therefore the number of signals observed in the $^{13}$C NMR is less than the number of C atoms in the related compounds **2a**−**c**, **5b**, and **6b**. While the $^{1}$H NMR spectra of the chloromethylated calix[*n*]arenes **1a**−**c**, **5a**, and **6a** show one singlet peak assigned to chloromethyl protons around 4.48 ppm, the same peaks are observed at 3.00 ppm attributed to the phosphonomethylene protons for compounds **2a**−**c**, **5b**, and **6b** as a doublet ($J_{PH}$ = 21.1 Hz).[13] The new peak for phosphoester derivatives **2a**−**c**, **5b**, and **6b** is seen as a doublet ($J_{POCH_3}$ = 10.0 Hz) around 3.60 ppm to 3.63 ppm attributed to the phosphonomethylester protons. These data show that the phosphorylation reaction of chloromethylated compounds **1a**−**c**, **5a**, and **6a** is completed. Furthermore, the presence of the phosphonate groups of phosphoester derivatives **2a**−**c**, **5b**, and **6b** was confirmed by $^{31}$P resonance signals around 29.00 ppm for phosphoester derivatives **2a**−**c**, **5b**, and **6b** ($CDCl_3$).

**Scheme 2. Synthetic Route for the Synthesis of Compounds 5c and 6c**[a]




[a] Reaction conditions: (i) trimethylphosphite, chloroform, reflux; (ii) BTMS and methanol, rt; (iii) 0.05 M NaOH.

**Liquid**−**Liquid Extraction Studies.** *Furosemide.* Furosemide is a derivative from the anthranilic acid, whose structure is presented in Figure 2, and represents a powerful loop diuretic that is widely used in the treatment of hypertension and edema. It is usually commercialized as tablets or parenteral solutions. The orally bioavailability of furosemide is very poor due to aqueous solubility at gastrointestinal pH, making solubility the rate-determining step in the gastric absorption of furosemide.[27] Several techniques have been used to increase its aqueous solubility, including cyclodextrin complexation.[31,32] Obtained results show that furosemide drug molecules are encapsulated into the hydrophobic cavity of cyclodextrins and a significant increase in the solubility and dissolution rate of furosemide. Also, calixarene compounds might form host−guest complexes with furosemide. Therefore, we have performed some preliminary evaluations to investigate binding efficiencies of the extractants **4a**−**c**, **5c**, and **6c** for furosemide by using solvent extraction. The results showed that furosemide could be extracted from organic phase into aqueous phase at neutral pH values. The results were summarized in Table 3. From Table 3, it is clear that the maximum extraction percentage occurs at 33.6 % for **5c**, 27.6 % for **6c**, and 21.5 % for **4a** toward furosemide. From the extraction data, water-soluble *p*-phosphonate derivatives **5c** and **6c** were found to be effective extractants for the phase transfer of furosemide. This interaction is attributed to the weak interaction forces including hydrogen bonding, $\pi-\pi$ interactions, dipole−dipole bonding or electrostatic interaction between hydrophobic cavity or alky phosphonate groups of receptors and phenyl, furan ring, or substituted group of furosemide. With the help of one or a combination of these forces furosemide most

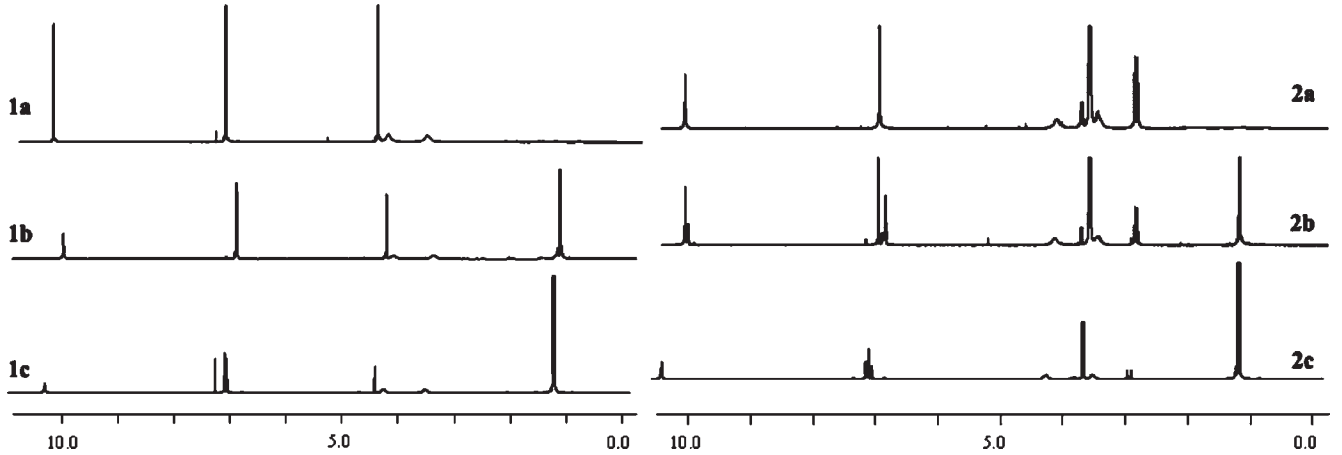



**Figure 1.** $^{1}$H NMR spectra of the chloromethylated (**1a**−**c**) and phosphorylated (**2a**−**c**) calix[4]arenes.

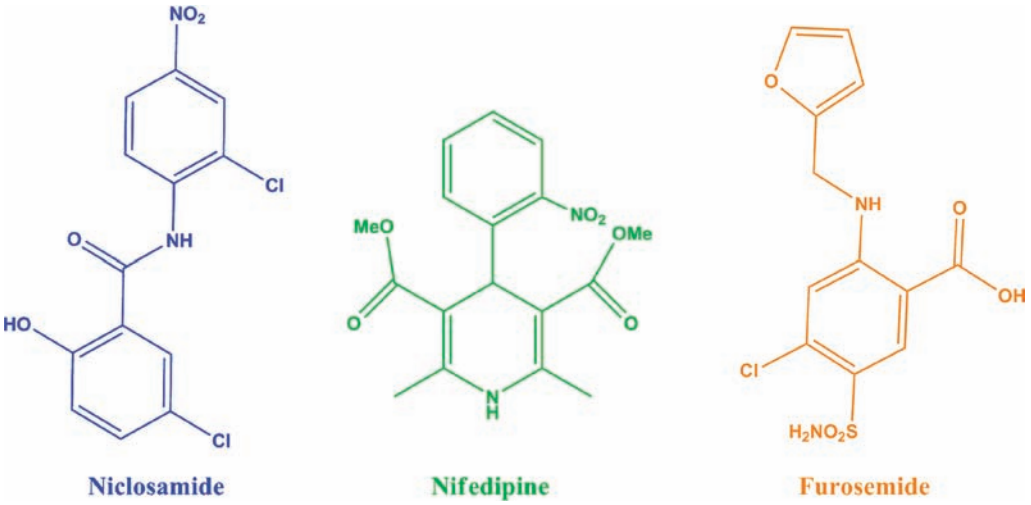



**Figure 2.** Molecular structures of drugs.

**Table 3. Extraction Percentage of Drug Molecules by Receptors**[a,b]

| compound | nifedipine | niclosamide | furosemide |
|---|---|---|---|
| **4a** | 11.3 ± 0.1 | 19.9 ± 0.1 | 21.5 ± 0.1 |
| **4b** | 13.1 ± 0.1 | 16.4 ± 0.1 | 19.8 ± 0.1 |
| **4c** | 5.1 ± 0.1 | 4.7 ± 0.1 | 6.1 ± 0.1 |
| **5c** | 7.3 ± 0.1 | 38.8 ± 0.1 | 33.6 ± 0.1 |
| **6c** | 43.3 ± 0.1 | 21.5 ± 0.1 | 27.6 ± 0.1 |
| blank | < 4.1 ± 0.3 | < 3.5 ± 0.3 | < 1 ± 0.3 |

[a] Averages and standard deviations calculated for data obtained from three independent extraction experiments. [b] Aqueous phase [ligand]: $4 \cdot 10^{-3}$ mol·kg$^{-1}$; organic phase: [drug molecule]: $1.35 \cdot 10^{-4}$ mol·kg$^{-1}$; at 298 K, for 5 h.

probably formed noncovalent inclusion complexes with the *p*-phosphonate calix[*n*]arenes similar to the complexes it forms with 4-sulfonic calix[*n*]arenes. 4-Sulfonic calix[6]arene improved the solubility of furosemide the most at 104 % followed by 4-sulfonic calix[8]arene at 102 %, while 4-sulfonic calix[4]arene increased the solubility of furosemide the least 81 %.[27]

*Nifedipine.* Nifedipine as a L-type calcium-channel blocker is used extensively for the clinical management of a number of cardiovascular diseases such as essential hypertension, congestive heart failure, and cerebral ischemia.[33] A major pharmaceutical problem associated with nifedipine is its poor aqueous solubility, 5 $\mu$g·cm$^{-3}$ to 6 $\mu$g·cm$^{-3}$ over a pH range of 2 to 10, which may account for its highly variable bioavailability in humans.[34] The extraction results obtained showed that nifedipine drug molecule could be extracted from organic phase into aqueous phase as shown in Table 3. The extractibility of nifedipine was not significantly changed with *p*-phosphonate calix[4]arene **4c** or calix[6]arene **5c** but did significantly increase with *p*-phosphonate calix[8]arene **6c**. Comparing the *p*-phosphonate calix[4]arene **4a**−**b** and *p*-phosphonate calix[8]arene **6c**, obtained results show that *p*-phosphonate calix[8]arene **6c** is a suitable extractant for nifedipine drug molecule. This is not a surprising result because it is expected that the larger calix[8]arene cavity would geometrically be more suited for effectively interaction with nifedipine than the smaller cavity of calix[4]arene (Figure 3).[25] In literature,[25] 4-sulfonic calix[8]arene improved the solubility of nifedipine, while in the presence of 4-sulfonic calix[6]arene, the solubility of nifedipine was decreased. Calix[8]arenes are more flexible than the calix[4]arenes owing to stronger intramolecular hydrogen bonding in the calix[4]arenes.[8] The strongest intramolecular hydrogen-bonding of calix[4]arenes would reduce its ability to form hydrogen bonds with nifedipine. Furthermore, free intra-annular OH groups of calix[*n*]arenes could involve hydrogen bonding between the phenolic hydrogen atoms of the calix[*n*]arenes and substituted groups of nifedipine. From Table 3, it is clear that maximum extraction percentage occurs at 43.3 % for **6c**, 11.3 % for **4a**, and 13.1 % for **4b** toward nifedipine. In the same time, weak interaction forces such as $\pi-\pi$ interactions, dipole−dipole bonding, and/or electrostatic attraction as mentioned above for furosemide may be another important contribution to the interaction between receptors **4a**−**b** and **6c** and nifedipine.

*Niclosamide.* Niclosamide is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog tapeworm.[2] This drug is also used as a molluscicide for the treatment of water in schistosomiasis control programs.[26] Niclosamide is practically insoluble in water (230 ng·cm$^{-3}$), which may severely limit its efficacy.[35] From the extraction results, it was observed that the niclosamide drug molecule could be extracted from the organic phase into the aqueous phase (Table 3). Comparing the extraction efficiency of calix[n]arenes, it was seen that phosphonate calix[6]arene **5c** was an effective extractant for niclosamide. From the extraction experiments, it was observed that the extraction percentage occurred at 21.5 % for **6c**, 38.8 % for **5c**, 19.9 % for **4a**, and 16.4 % for **4b** toward niclosamide. Niclosamide is a highly hydrophobic molecule as nifedipine and furosemide. Therefore, similar interactions to transport the drug molecule between *p*-phosphonate calix[*n*]arenes and niclosamide drug molecule probably occurred as mentioned in both nifedipine and furosemide. In literature[26] the water solubility of niclosamide increased with an increase in the concentration of 4-sulphonato-calix[*n*]arenes, but as the concentrations increased additionally, the solubility dropped to a lower level, indicating that insoluble complexes were formed at higher concentrations. It is expected that the larger cavities would geometrically be more suited for a stronger interaction with niclosamide.[26] However, calix[8]arenes have less effect than calix[6]arenes. This situation might be attributable to the dimension of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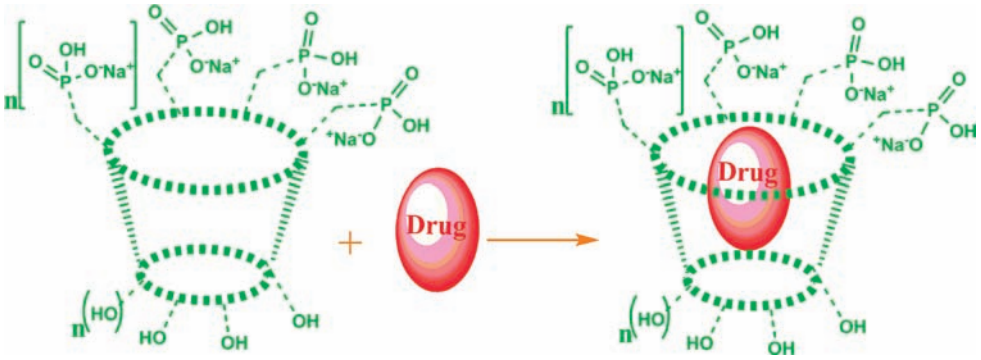


**Figure 3.** Proposed interaction of calixarenes with drug molecule ($n$: 1, 3, or 5).

calix[6]arene which is most optimal for the niclosamide molecule, because "host-size selectivity" does exist in host−guest-type complexation with calixarenes.[8,20] Hydrogen bonding and weak interaction forces as $\pi-\pi$ interactions and dipole−dipole bonding are the most important forces responsible for the extraction of niclosamide. *p*-Phosphonate calix[*n*]arenes contain free intraannular OH groups, suggesting that the complexation mechanism could involve hydrogen bonding between the phenolic hydrogen atoms of the calix[*n*]arenes and the substitute group of niclosamide.

## CONCLUSIONS

In the course of this study, several phosphorylated calix[*n*]arene receptors were easily synthesized in large quantities and converted their corresponding water-soluble *p*-phosphonate derivatives. Also, drug extraction studies from the organic phase to the aqueous phase were performed. The complexation studies showed that compounds **4a**−**c**, **5c**, and **6c** were effective receptors for niclosamide, furosemide, and nifedipine drug molecules. It could be concluded that the complexation of drug molecules depends on the structural properties of the water-soluble *p*-phosphonate calix[*n*]arene such as hydrophobic cavity diameters, hydrogen binding ability, stability or rigidity, and also depends on ion−dipole attraction or electrostatic interaction between *p*-phosphonate calix[*n*]arene and drug molecules. Further investigations to understand the use of compounds **4a**−**c**, **5c**, and **6c** as drug soluble agents toward niclosamide, furosemide, and nifedipine are currently in progress.

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