# Preparation and Evaluation of *p-tert*-Butyl-Calix[4]arene-Bonded Silica Stationary Phases for High Performance Liquid Chromatography

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## Key Words

Column liquid chromatography
*p-tert*-Butyl-calix[4]arene-bonded silica
Nuclear magnetic resonance spectrometry
PAHs
Nucleosides bases

## Summary

A new method is proposed for preparation of a *p-tert*-butyl-calix[4]arene-bonded silica stationary phase. The chemically modified *p-tert*-butyl-calix[4]arene is attached to silica gel via the silane coupling reagent γ-(ethylenediamino)-propyl-triethoxyl-silane. The bonded phase has been characterized by $^{29}Si$ and $^{13}C$ cross polarization/magic angle spinning solid-state nuclear magnetic spectrometry. The retention behavior of polycyclic aromatic hydrocarbons (PAHs), nucleosides and bases has been investigated on the bonded phase in the reversed-phase mode.

## Introduction

Calixarenes[1] are stable cyclic oligomers composed of phenolic units linked by methylene bridges at positions ortho to the hydroxyl groups, building up beakershaped hydrophobic cavities at the upper rim and hydrophilic phenolic hydroxyl groups at the lower rim. As host molecules with a specific structure, calixarenes represent a third generation of excellent host molecules following crown ethers and cyclodextrins in supramolecular chemistry [2]. Calixarenes possess the capability to include neutral organic molecules like cyclodextrins and the capability of chelating and transporting cations like crown ethers. Furthermore, parent calixarenes are very useful building blocks for various novel acceptors with preogranized structure which can be obtained by introducing different functional groups. Crown ethers and cyclodextrins have been applied in gas chromatography and liquid chromatography as selective phases for a number of years [3–6]. Their chromatographic behavior is chiefly connected with their inclusion complexing properties with many neutral organic compounds and ions. Although some applications of calixarenes have been reported, such as ion selective electrodes [7], recovery of metal ions [8] and phase transfer agents [9], little work has been carried out on the utility of calixarenes in chromatography. Friebe et al. [10] have reported the use of *p-tert*-butylcalix[4]arene-bonded silica gel stationary phase in the liquid chromatographic separation of nitroaniline, regioisomers, nucleosides and three proline-containing dipeptides. Glennon et al. [11] have prepared a silica-bonded calix[4]arene tetraester stationary phase and used it in the liquid chromatographic separation of amino esters and alkali metal ions. We have reported that the chromatographic separation of polycyclic aromatic hydrocarbons (PAHs), nucleosides and some disubstituted aromatic positional isomers on a *p-tert*-butyl-calix[6]arene-bonded silica stationary phase [12]. Recently, Park et al. [13] have successful separated some mono-substituted phenol and some other aromatic positional isomers on a calix[6]arene-*p*-sulfonate-bonded silica stationary phase. Mangia et al. [14] have, for the first time, reported the separation of alcohols, chlorinated hydrocarbons and aromatic compounds by gas-chromatography with *p-tert*-butylcalix[8]arene on Chromosorb. Utilization of calix[n]arene (n = 4,5,6,8) [15, 16], rescorcin[4]arenes [17, 18] and calixcrowns [19] in gas chromatography has also been reported.

Some methods have been proposed for structure characterization of the bonded-silica stationary phases. Weight gain analysis, elemental analysis and IR spectra analysis are usually adopted for the purpose. However, these methods generally afford uncertain information of the stationary phases. Recently, $^{13}C$ solid state NMR has become a powerful tool for the structure characterization of the bonded-silica stationary phase [20–22].



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In this paper, we describe how to synthesize a new *p-tert*-butyl-calix[4]arene-bonded silica stationary phase by a new easy route and the assignment of all the peaks of $^{13}C$ solid state NMR. Chromatographic separation of PAHs, nucleosides and bases with this phase is also described.

## Experimental

### Chemicals and Reagents

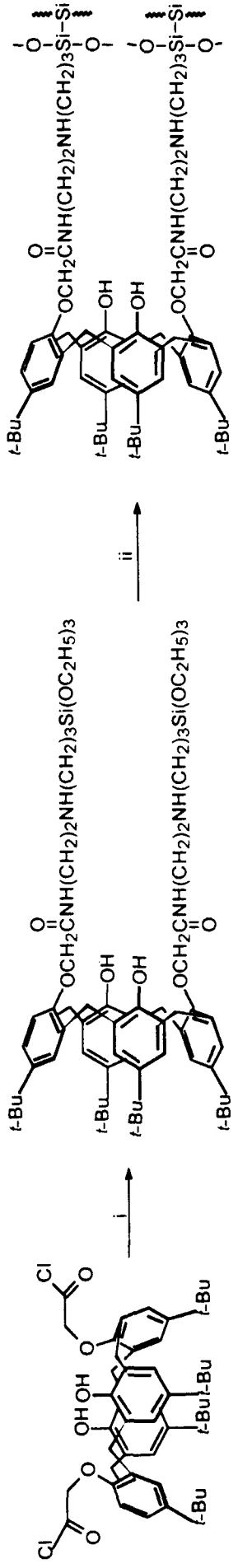
Silica (5–7 μm particle size) was purchased from Qingdao Ocean Chemical Plant (Qingdao, China); γ-(ethylenediamino)-propyl-triethoxyl-silane (content ≥ 90 %) was purchased from Wuhan University Chemical Plant (Wuhan, China); 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(chloroformylmethoxyl)-26,28-dihydroxylcalix[4]arene (**1**) was prepared according to a reported procedure [23]; other reagents were AnalaR grade unless indicated otherwise .

### Synthesis of *p-tert*-Butyl-Calix[4]arene-Bonded Silica Stationary Phase

The silica gel-linked macrocycles were synthesized in three steps starting from (**1**) prepared according to the published procedure [23] (see Scheme 1). First, a mixture of compound (**1**) γ-(ethylenediamino)-propyl-triethoxyl-silane and triethylamine in 150 mL of dry toluene was stirred at room temperature under a nitrogen atmosphere for 16 h, after which 2.2090 g silica gel was added. The mixture was stirred and heated under reflux for 24 h under dry $N_2$. The solid material was filtered and washed in sequence with toluene, acetone, methanol and distilled water for three times and the *p-tert*-butylcalix[4]arene-bonded silica phase was dried at 120 °C for 3 h, then cooled to room temperature in a desiccator. Weight gain of the product relative to the original silica was 52.98 %.

### CP/MAS NMR Spectroscopy

The measurements of $^{29}Si$ CP-MAS (cross polarization/magic angle spinning) NMR were performed on a Bruker MSL400 spectrometer on the *p-tert*-butylcalix[4]arene-bonded silica and basic silica in a rotor of zirconium oxide. The magic-angle spinning was carried out at a rate of 4000 Hz. The spectra were recorded with a pulse length of 5.5 μs and a contact time of 4 ms. 400 scans were accumulated with a repetition rate of 1 s for each spectrum. $^{13}C$ CP-MAS NMR measurements were performed on a home-assembled 200 MHz spectrometer on the *p-tert*-butylcalix[4]arene-bonded silica in a zirconium oxide rotor at a rate of 3200 Hz. The spectrum was recorded with a pulse length of 3 μs and a contact time of 1 ms. 10000 scans were accumulated with a repetition rate of 1 s. All NMR spectra were externally referenced to liquid tetramethylsilane.




**Scheme 1**
Synthesis of *p-tert*-butyl-calix[4]arene-bonded silica stationary phase: (i) γ-(ethylenediamino)-propyl-triethoxyl-silane, $Et_3N$ and toluene under reflux; (ii) silica gel, $Et_3N$ and toluene under reflux.

### Chromatographic Procedure

The chromatographic system consists of a Model LC-10AD pump (Shimadzu, Japan), a Reodyne 7125 injector with 20 μL loop, a Model SPD-10A (Shimadzu, Japan), UV-Vis spectrophotometric detector, and a type 3066 pen recorder (Sichuan the fourth instrument plant, Sichuan, China). The *p-tert*-butylcalix[4]arene-bonded silica stationary phase was slurry-packed into a 15 cm × 4.6 mm (i.d.) stainless-steel column. The column temperature was controlled at 28 ± 0.2 °C. An aqueous buffer solution of 0.02 mol $L^{-1}$ $NaH_2PO_4$ (pH 3.5) was used as the mobile phase for nucleosides and bases and a mixture of methanol-water (60:40,v/v) for PAHs. Before use, the mobile phases were generally filtered through a G-4 filter glass funnel and degassed in an ultrasonic bath for 5 min. The flow rate was set at 1.0 mL $min^{-1}$. The samples were dissolved in methanol. The wavelength for detection was 254 nm.

## Results and Discussion

### Synthesis of *p-tert*-Butyl-Calix[4]arene-Bonded Silica Stationary Phase

Several approaches have been proposed for preparation of calixarene-bonded silica stationary phases [11, 13]. Trifunctional silane containing calixarene, synthesized through a hydrosilation addition reaction, has been grafted to silica gel [11]. However, the hydrosilation addition reaction gives a low yield. Another method is to synthesize calixarene derivative with highly reactive functional groups, which are then attached to the silica activated with a silane coupling reagent [13]. In this method, the yield of the last step of the synthesis was very high, but getting the calixarenes derivative faces many obstacles including synthesis and separation of a derivative. In previous work [12], we have reported that the parent *p-tert*-butyl-calix[6]arene reacts with the silane coupling reagent 3-glycidoxypropyltriethoxysilane to open the epoxy ring, and then reacts with silica gel for preparation of *p-tert*-butyl-calilx[6]arene-bonded silica phase without any further purification. The method is easy and convenient, but elemental analysis showed a low content of *p-tert*-butyl-calix[6]arenes immobilized to the silica gel. Scheme 1 shows a new route for synthesizing a *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase. In this procedure, no separation and purification are required, except for washing the final product. The introduction of chloroformyl groups to *p-tert*-butyl-calix[4]arene causes it to react easily with a silane coupling reagent containing a primary amine. The silane coupling reagent containing *p-tert*-butyl-calix[4]arene is then attached to the silica gel. The fine structure of the resulting boned phase is characterized by means of solid state NMR spectroscopy.

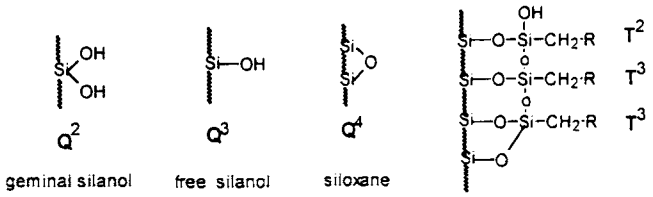



**Figure 1**
The various types of groups likely to be found on the surface of calix[4]arene-bonded silica.

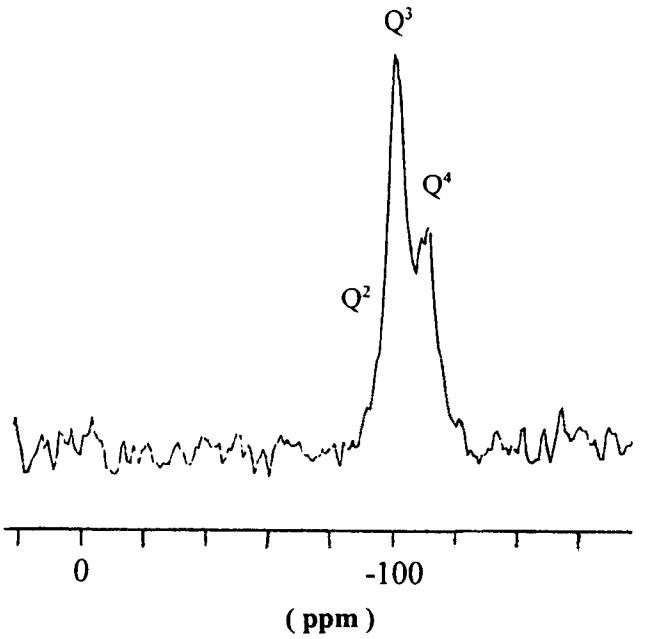



**Figure 2**
$^{29}Si$ CP-MAS NMR spectrum of a basic silica gel.

### CP/MAS NMR Spectroscopy

Different species of silicon groups on the surface and typical $^{29}Si$ CP/MAS NMR spectra of a basic silica gel and a chemically modified silica gel have been reviewed in detail by Albert et al. [21]. The $^{29}Si$ CP/MAS NMR spectra for a basic silica gel and a *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase are shown in Figure 2 and Figure 3, respectively. As shown in Figure 2, two strong signals at –100 and –110 ppm correspond to $Q^3$ and $Q^4$, respectively (see Figure 1 [20]). But the signal of $Q^2$ was not clear even when changing the contact time and repetition rate over a wide range, indicating that the amounts of the geminal silanol on the surface of the silica are very small. In Figure 3, two very strong signals at –56 and –65 ppm corresponded to $T^2$ and $T^3$ as shown in Figure 1, suggest that a large proportion of the trifunctional alkylsilyl groups have been crosslinked to the surface of the silica gel. Comparing to Figure 2, the relative strength of $Q^3$ shown in Figure 3 became smaller, which shows that after chemical modification the number of free silanols decrease drastically and the number of siloxane groups increase.

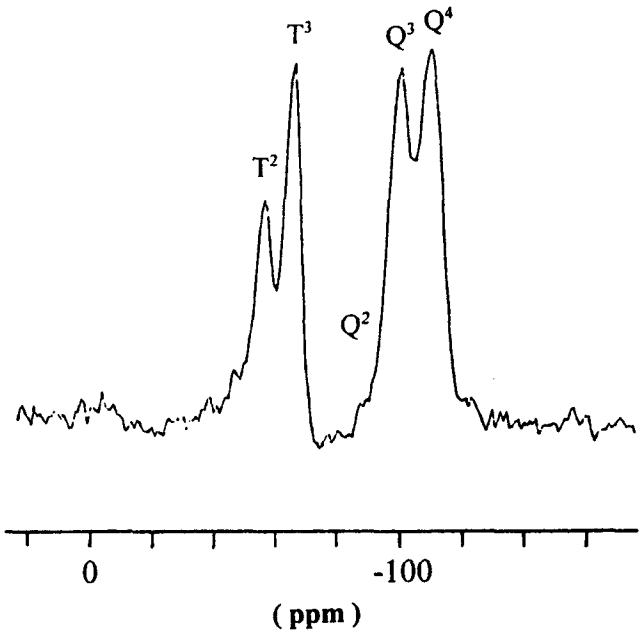



**Figure 3**
$^{29}$Si CP-MAS NMR spectrum of the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase.

The $^{13}$C CP-MAS NMR spectrum of the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase is shown in Figure 4 and all the carbon atoms and their signals are labeled. As shown in Figure 4, the signals of most carbon atoms on the alkyl chain and noncyclic carbon atoms are present in a higher field; the signals of the carbon atoms on the benzene ring and carbonyl carbon atoms on the alkyl chain appear in a lower field. The high intensity of the peaks in the aromatic region from 155 to 120 ppm show that the content of *p-tert*-butyl-calix[4]arenes on the surface of the silica gel is high. The starred peak at 170 ppm is due to rotary sidebands introduced by carbon atoms in the benzene ring, which was verified by changing rotation speed. Furthermore, the peaks for ethoxy groups of triethoxy alkylsilane have not been observed, which also affirmed bonding with a high degree of crosslinkage.

## Chromatographic Separation of PAHs, Nucleosides and Bases

The chromatographic performance of the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase was evaluated by using PAHs, nucleosides and bases as solutes. Figure 5 shows a typical chromatogram of a mixture of PAHs with methanol-water (60:40, v/v) as the mobile phase. It can be seen that the mixture of PAHs is separated completely and the elution order is the same as that obtained on ODS. This result implies that the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase has a reversed-phase chromatographic performance. Typical chromatograms of nucleosides and bases on the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase with 0.02 mol $L^{-1}$ $NaH_2PO_4$ (pH 3.5) buffer solution as mobile phases are shown in Figure 6 and Figure 7, respectively. These chromatograms show that partial separation of four nucleosides and six bases could be achieved even though most of the peaks are tailing. The elution order of nucleosides (C>U>A>G) as shown in Figure 6 was different from that obtained by Friebe et al. on a *p-tert*-butyl-calix[4]arene-bonded phase (C>U>G>A) [10], which is the same as that on an ODS column [24]. The difference of elution order on the two *p-tert*-butyl-calx[4]arene-bonded phases may be

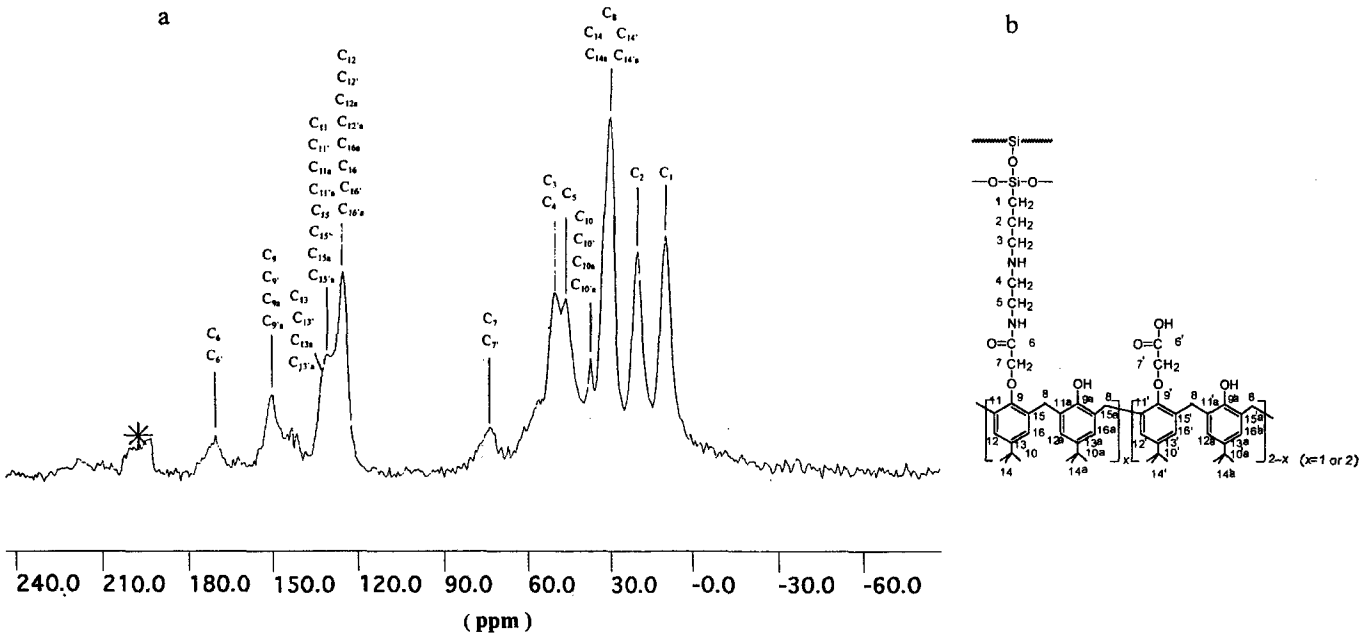



**Figure 4**
(a) $^{13}$C CP-MAS NMR spectrum of the *p-tert*-butyl-calix[4]arene-bonded silica gel stationary phase; (b) structure and assignment of the phase.

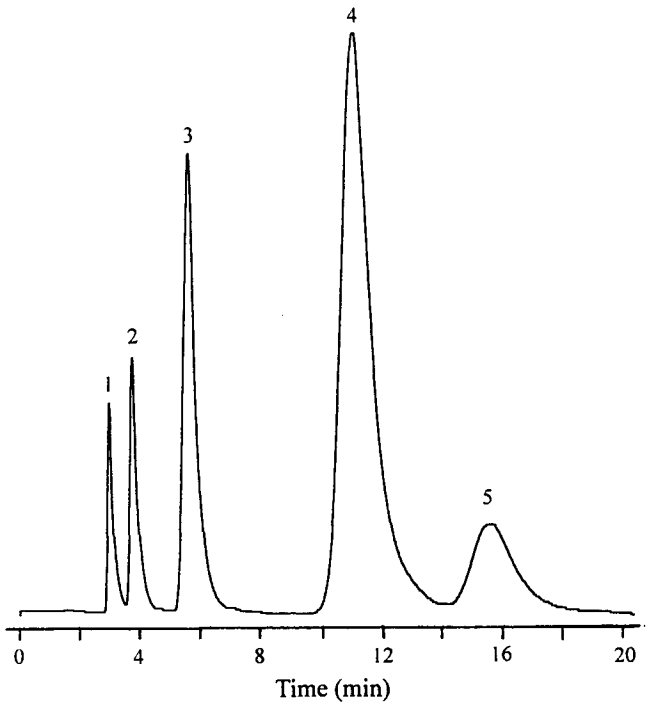



**Figure 5**
Typical chromatogram of a mixture of polycyclic aromatic hydrocarbons: mobile phase: methanol-water (60:40); flow rate: 1.0 mL $min^{-1}$; **1** = benzene; **2** = toluene; **3** = naphthalene; **4** = bihenyl; **5** = anthrancene.

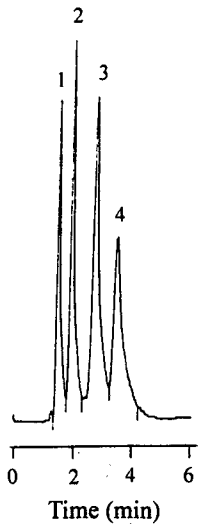



**Figure 6**
Typical chromatogram of a mixture of nucleosides: **1** = cytidine (C); **2** = uridine (U); **3** = guanosine (G); **4** = adenosine (A).

attributable to the difference in their space arms and amounts of the bonded *p-tert*-butyl-calix[4]arene.

Because it contained *p-tert*-butyl-calix[4]arenes, amine groups and carbonyl groups, the phase prepared in this study was a mixed-mode phase. Therefore, the retention mechanism of the solutes on the phase is complicated. Although *p-tert*-butyl-calix[4]arenes are a well-known host molecule, which can complex with small neutral molecules such as toluene in solid state [25] and derivatives have been reported to be able to include ions such as $Na^+$ [26], $UO_2^{2+}$ [27] and $Ag^+$ [28]. The cavity of the bonded *p-tert*-butyl-calix[4]arene was not big enough for nucleosides or bases used in this study. Therefore, the retention of nucleosides and bases on the phase must be ascribed to electrostatic interaction, π-donor-π-acceptor interaction, hydrogen bonding interaction, and partial inclusion interaction between the solutes and the stationary phase.

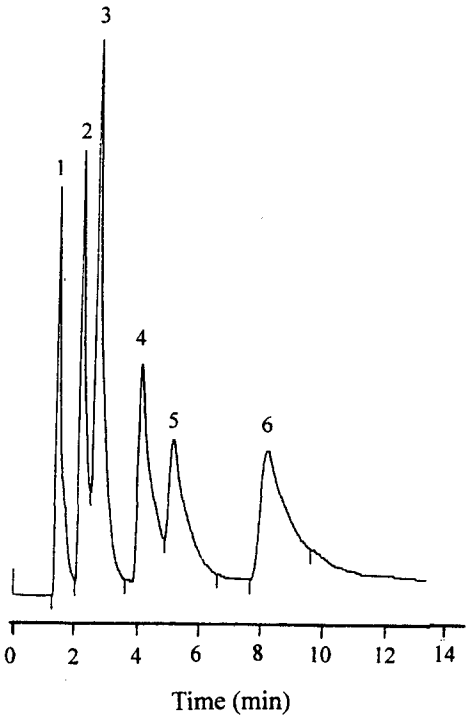



**Figure 7**
Typical chromatogram of a mixture of bases: **1** = cytosine; **2** = 5-flurouracil; **3** = purine; **4** = 5-iodouracil; **5** = 6-mercaptopurine; **6** = caffeine.

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## References

[1] a) *C. D. Gutsche*, in calixarenes, ed. *J. F. Scoddart*, The Royal Society of Chemistry, Cambridge, 1989; b) *V. Böhmer*, Angew. Chem., Int. Ed. Engl. **34**, 713 (1995).
[2] *S. Shinkai*, Tetrahedron, **49**, 8933 (1993).
[3] *D. W. Armstrong*, U. S. Patent 4539399 (1985).
[4] *D. W. Armstrong, C. D. Chang, S. H. Lee*, J. Chromatogr. **539**, 83 (1991).
[5] *D. W. Armstrong, A. M. Stalcup, M. L. Hilton, J. D. Duncan, J. R. Faulkner, Jr., S. C. Chang*, Anal. Chem. **62**, 1610 (1990).

[6] *S.-L. Da, W.-H. Yue, Y.-F. Wen, H.-L. Da, Z.-H. Wang*, Anal. Chim. Acta **299**, 239 (1994).
[7] *D. Diamond, G. Svehla, E. M. Seuard, M. A. McKervey*, Anal. Chim. Acta **204**, 223 (1998).
[8] *S. R. Izatt, R. T. Hawkins, J. J. Christensen, R. M. Izatt*, J. Am. Chem. Soc. **107**, 63 (1985).
[9] *H. Taniguchi, E. Nomura*, Chem. Lett. **1773** (1998).
[10] *S. Friebe, S. Gebauer, G. J. Krauss*, J. Chromatogr. Sci. **33**, 281 (1995).
[11] *J. D. Glennon, E. Horne, K. O'Connor, G. Kearney, S. J. Harris, M. A. McKervey*, Anal. Proc. **31**, 33 (1994).
[12] *W. Xu, J.-S. Li, Y.-Q. Feng, S.-L. Da, Y.-Y. Chen, X.-Z. Xiao*, Chromatographia **48**, 245 (1998).
[13] *Y. K. Lee, Y. K. Ryu, J. W. Ryu, B. E. Kim, J. H. Park*, Chromatographia **46**, 507 (1997).
[14] *A. Mangia, A. Pochini, R. Ungaro, G. D. Andreetti,* Anal. Lett. **16**, 1027 (1983).
[15] *P. Mñuk, L. Feltl,* J. Chromatogr. A, **696**, 101 (1996).
[16] *P. Mñuk, L. Feltl, V. Schurig*, J. Chromatogr. A **732**, 63 (1996).
[17] *H.-B. Zhang, Y. Ling, R.- J. Dai, Y.-X. Wen, R.-N. Fu, J.-L. Gu*, Chem. Lett. 225 (1997).
[18] *D.-Q. Xiao, Y. Ling, Y.-X. Wen, R.-N. Fu, J.-L. Gu, R.-J. Dai, A.-Q. Luo*, Chromatographia **46**, 177 (1997).
[19] *Z.-L. Zhong, C.-P. Tan, C.-Y. Wu, Y.-Y. Cheng*, J. Chem. Soc., Chem. Commun. 1737 (1995).
[20] *R. Brindle, K. Albert, S. J. Harris, C. Tröltzsch, E. Horne, J. D. Glennon*, J. Chromatogr. **731,** 41 (1996).
[21] *K. Albert, E. Bayer*, J. Chromatogr. **544**, 345 (1991).
[22] *E. Bayer, K. Albert, J. Reiners, M. Nieder, D. Müller*, J. Chromatogr. **268**, 197 (1983)
[23] *E .M. Collins, M. A. McKervey, E. Madigan, M. B. Moran, M. Owens, G. Ferguson, S. J. Harris*, J. Chem. Soc. Perkin Trans. I, 3137 (1991).
[24] *R. A. Hartwick, S. P. Assenza, P. R. Brown*, J. Chromatogr. **186**, 647 (1979).
[25] *G. D. Andreetti, R. Ungaro, A. Pochini*, J. Chem. Soc., Chem. Commun. 1005 (1979).
[26] *E. M. Collins, M. A. McKervey, S. J. Harris*, J. Chem. Soc., Perkin Trans. I, 372 (1989).
[27] *S. Shinkai, H. Koreishi, K. Uedo*, J. Am. Chem. Soc. **109**, 6371 (1987).
[28] *W. I. I. Bakker, W. Verboom, D. N. Reinhoudt*, J. Chem. Soc., Chem. Commun. 71 (1994).

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