2005

Identification and Recovery of an Asymmetric Calix[4]arene Tetranitrile Derivative using Liquid Chromatography and Mass Spectrometry

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Recommended Citation

Schazmann, B.(2005) Identification and recovery of an asymmetric calix[4]arene tetranitrile derivative using liquid chromatography and mass spectrometry. *Supramolecular Chemistry,* 2005, 17, 393-399. DOI:10.1080/10610270500138789

Funder: Enterprise Ireland, grant code SC/2002/161, Science Foundation Ireland support under the Adaptive Information Cluster award (SFI03/IN3/1361)

Identification and recovery of an asymmetric calix[4]arene tetranitrile derivative using liquid chromatography and mass spectrometry.

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Abstract

A simple analytical LC-MS (Liquid Chromatography Mass Spectrometry) method and associated instrumentation has been adapted for use by the organic chemist to yield mg quantities of target compound from a reaction mixture.

Calix[4]arene **3** was identified as representing 51% of total peak area of a reaction mixture containing no less than 10 components, using LC-MS. This peak corresponded to a mass of 878.8, equivalent to a complex of **3** and an ammonium cation. Molecular models further rationalise this observation by showing that the asymmetric binding cavity of **3** is suitable for binding tetrahedral guests like the ammonium ion.

By scaling up the LC method, using analytical instrumentation, 55mg of 98% pure **3** were isolated with a recovery yield of 90% in 1 hr.

The current method represents a powerful and easily adapted tool for monitoring a challenging synthesis which combines identification, efficient separation and partial characterisation for reaction mixture components using readily available instrumentation and methods.

Keywords: calixarene, HPLC, MS, semi-preparative, isolation.

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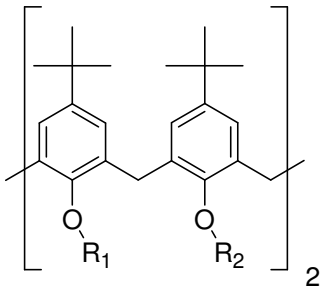
Introduction: Structures **1-6**, in Figure 1, belong to a large family of compounds called calixarenes. There are numerous compounds based on the calixarene molecular platform which all have certain features in common, namely a central aromatic cavity or annulus, an upper rim and a lower rim, substituted as required. Substitution often locks the calixarene into a rigid cup like cone conformation, ideal for selective host recognition. In short, calixarenes make excellent platforms for the design of chemical sensor receptors for ions and neutral molecules. Several excellent publications are available describing the history, synthesis and characteristics of calixarenes[1-4].

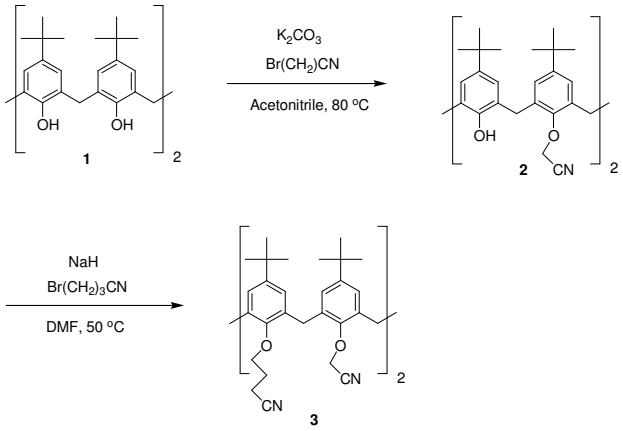


Calixarene	R_1	R_2
1	H	H
2	H	CH_2CN
3	$(CH_2)_3CN$	CH_2CN
4	$(CH_2)_3CN$	$(CH_2)_3CN$
5	$CH_2C(O)_2Et$	$CH_2C(O)_2Et$
6	$CH_2C(O)_2Et$	$C_2H_4C(O)_2Et$

Figure 1. Structural formulae of calix[4]arenes.

To date, the most commercially successful sensor calixarenes are *symmetrically* substituted calix[4]arenes like the tetraester **5**, which acts as a selective sodium host in chemical sensor applications[4].

Scheme 1. Synthesis of 3.



Calix[4]arene **3** was synthesised as shown in Scheme 1. Structure **3** was envisaged as a precursor of a host for non-spherical shaped cations and anions. This is due to the differing lengths in the alternate pendant groups represented in the spatial arrangement of functionality on the lower rim. One possible low energy conformation is that shown in Figure 2. Evidence for this cone conformation are the two doublets observed at 4.33 and 3.28ppm for the methylene protons in the calixarene's annulus, as seen in the proton NMR of **3**[1]. It must be acknowledged that the current model's lower rim pendant groups must have considerable flexibility due to the methyl and propyl spacers.

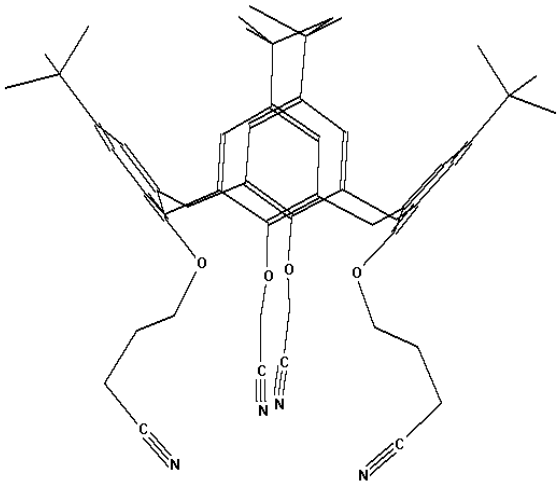


Figure 2. Energy Minimised model of 3 generated using MM2 in Chem 3-d Pro v.8.0 (Cambridgesoft Corporation).

The mixture from the synthesis of **3** was analysed by LC-MS. The peak labelled **(3)** in Figure 3 corresponds to a molecular ion +m/e 878.8 ([M + NH_4^+], calcd 878.6) with an area of 51% relative to total peak area. Ammonium ions, presumably originating from the synthetic workup, appeared to stabilise the molecule **3**.

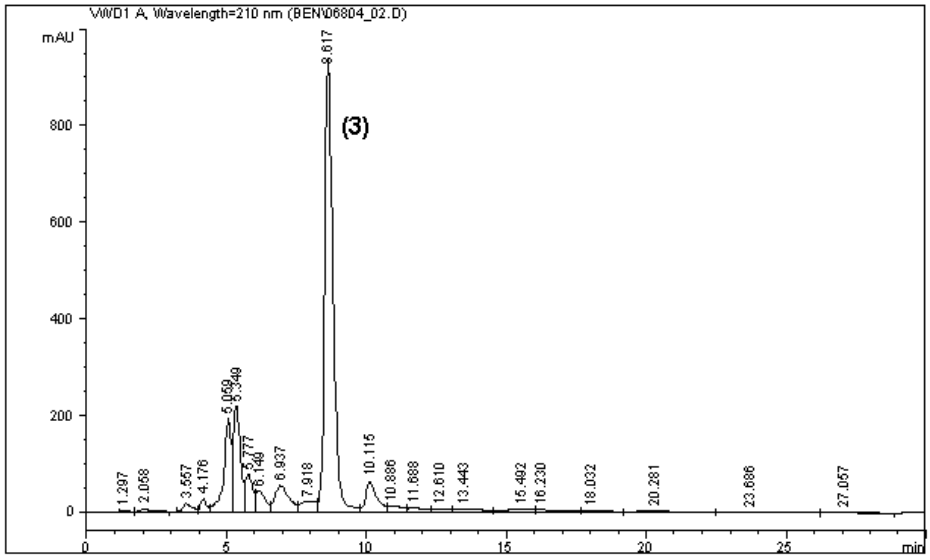


Figure 3. LC chromatogram of reaction mixture; 3 identified by MS.

This observation suggests that **3** may be predisposed towards binding tetrahedrally shaped cations due to the spatial arrangement of its binding sites. Preliminary potentiometric screening of **3** revealed significant responses towards a number of cations, confirming that **3** is energetically capable of spontaneous complex formation. Potentiometric membranes were prepared by a well established method[5]. Table 1 depicts the potential change of a PVC membrane containing **3** when in contact with a 10^{-1}M aqueous solution of the indicated cation compared to equivalent measurements in deionised water. This demonstrates that complex formation is indeed occurring with a particular order of selectivity, which favours ammonium and potassium over sodium. Further potentiometric work is in progress and will be the subject of a separate report which is currently in preparation.

Cation	Potential change (mV)
K^+	+216.0
NH_4^+	+206.9
Na^+	+154.0
Ca^{2+}	+79.4
Li^+	+71.4
Mg^{2+}	+62.4

Table 1. Potential changes of potentiometric membrane containing 3 in contact with 10^{-1}M aqueous solutions of the indicated cation chloride, compared to the equivalent potential in deionised water.

Sodium selectivity often appears as the 'default' selectivity when cation selective t-butyl calix[4]arenes are experimentally assessed[6]. This is due to the excellent fit of the sodium cation with the cavity of many calix[4]arenes, such as **5** for example[4,7]. The cavity of **5** is defined by localised electron density of four phenoxy and four carbonyl oxygen atoms. In contrast, for compounds such as **3** and **4**, cations appear to interact with the nitrile functional groups, with less involvement of the phenoxy oxygens: Figure 4 shows the interaction of ammonium (for illustrative purposes) with **3** and **4**. This complexation arrangement appears to be similar for all common earth and alkali earth metals. The nitrile groups may offer an alternative binding pocket further removed from lower rim defined, that involves the phenoxy oxygen atoms to a lesser extent, and this may also lead to radically different selectivity in ion binding behaviour. Cation interaction further from the annulus is supported by preliminary results from potentiometric investigations of **3**, showing a selectivity for ammonium and potassium over sodium, and these larger cations are not optimally accommodated at the geometrically restricted region of the calix[4]arene lower rim. The ammonium cation is of particular interest due to geometric complementarity with the spatial arrangement of the nitrile groups of **3**, and the potential for hydrogen bonding with these binding sites.

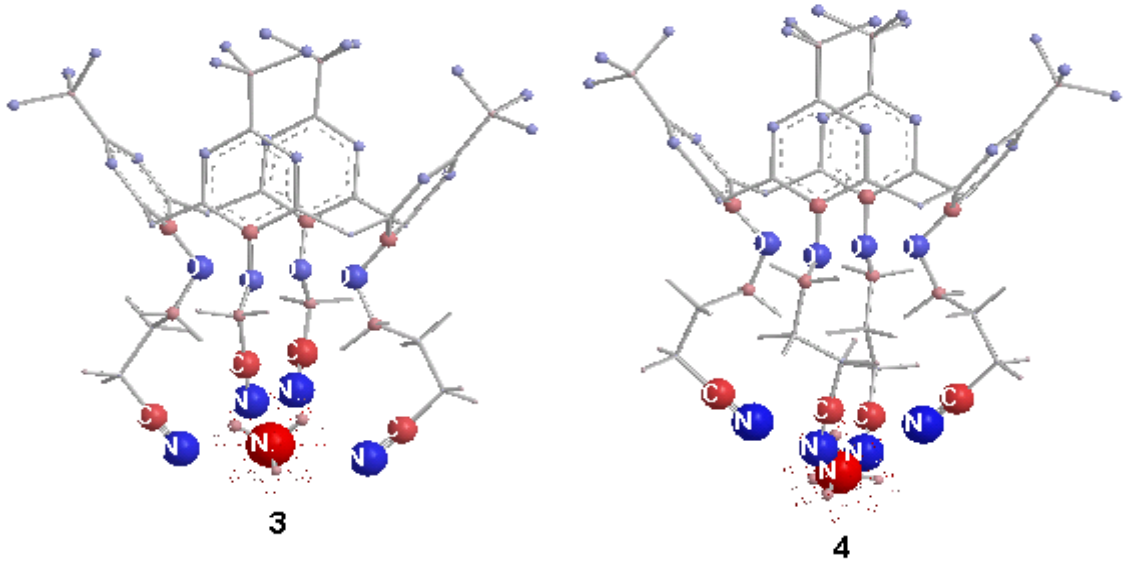


Figure 4. Complexes of 3 and 4 with an ammonium ion. Atoms are scaled by size according to Huckel partial charges. Red and blue are areas of positive and negative localised charge respectively. The importance of the nitrile groups for complexation is revealed.

We have been interested in *asymmetrically* substituted compounds such as **3** because of the possibility of generating ligands with dramatically different ion-binding selectivities than previously observed. However, the synthetic route to these derivatives has proven more difficult than anticipated. For example, starting from **1**, we attempted to make the asymmetric derivative **6**, that has an additional methylene spacer in adjacent pendant ester groups. However, we found that due to (a) the high degree of substitution, (b) increasing difficulty to deprotonate successive phenol hydrogens and (c) the sterically hindered nature of target compounds, yields obtained were very small or the reactions were unsuccessful. Analogously, the synthesis of **3**, as seen in Scheme 1, did not proceed as readily as that of **4**[8]. This prompted the use of more advanced work up tools.

Normal phase flash chromatography or open-column chromatography have been used for many years for separations[9]. To date, these techniques have been sufficient to separate and recover calixarene compounds from each other in the majority of cases. However, we found that LC-MS was necessary for the separation and identification of

the more complex product profile obtained in these reactions. HPLC is a common analytical technique which generally has much better separating efficiency than open column or flash chromatography[10]. This improved efficiency can prove critical when dealing with a low yield of product in a complex mixture.

The use of HPLC for *preparative* purposes has largely been the preserve of industry since dedicated instrumentation can be expensive to acquire and run and as such, preparative HPLC has been perceived as a specialist technique by most organic research chemists. Dedicated preparative instrumentation and materials have been used for asymmetrically substituted calix[4]arenes without MS in the past[11], and several useful analytical HPLC methods have been developed[12]. While scaling up of an existing analytical method for use in preparative HPLC must be done with care, we have found that good results can often be achieved in a relatively straightforward manner. It is our view that the ease of extending the use of both *analytical* scale HPLC and MS for semi-preparative product characterisation, isolation and collection has not been sufficiently highlighted in the literature. In this report, we highlight the importance of using advanced analytical characterisation of reaction products coupled with semi-prep methods for scaling up the process in order to obtain reasonable quantities of more elusive derivatives such as the asymmetric calix[4]arene **3**.

Results and Discussion: The chromatogram in Figure 3 revealed the complex nature of the reaction mixture obtained for the synthesis of **3**. Numerous peaks or bands are present with considerable co-elution. **3** and some other components in the mix could be identified. Starting materials are seen at about 5 minutes, including **2**. Between 5 and 8.6 minutes (peak of **3**) are various breakdown fragments of **3**. Interestingly, molecular ions of **3**+K^+ were seen in this region too. An unidentified component of

greater mass than **3** was seen at about 10.1 minutes. The reverse phase nature of the column stationary phase ensures that components larger (and less polar) than **3** emerged after 8.6 minutes. By-products and un-reacted or partially reacted starting materials were therefore largely confined to retention times below 8.6 minutes.

With a rather complex reaction mixture containing **3** present, it was decided that the analytical HPLC method would be scaled up to isolate **3** as efficiently as possible, instead of resorting to open column chromatography for separation. If normal phase chromatography was used instead, the compound of interest would elute early and would be less likely to be resolved from related compounds. This scenario applies to the use of open column chromatography where silica gel is often used as a stationary phase.

Using a simple scale up factor as a guideline, supplied by most column manufacturers, HPLC parameters were altered for semi-preparative work. Column width went from 2.0mm to 10.0mm with a larger particle size of the same stationary phase. The stationary phase used for this work, Synergy Fusion-RP, has both reverse and normal phase characteristics meaning that a mixture with a broad range of polarities can be effectively separated in the one chromatogram, thus saving time and consumables.

Flow rate was increased from 0.2ml/min to 5ml/min. These flow rates correspond to a flow rate of 1ml/min on a more typical 4.6mm diameter column. The injector, pump, detector cell and tubing of most analytical HPLC hardware is capable of running at these conditions. Indeed, even with a flowrate of 5ml/min, the back pressure never rose above 33bar, well within the instrument's maximum limit.

The 100µl standard analytical injection loop was retained as it is more beneficial to increase injected sample concentration than volume for efficient separation[13]. Sample

concentration was increased for preparative work from 0.5mg/ml to 300mg/ml. Being aware that the UV detector cell was designed for analytical concentrations, it was decided to change the wavelength used for preparative work to one showing less sensitivity towards the sample, thus avoiding detector saturation. **3** absorbs about 6 times less UV radiation at 280nm than at 210nm. The analytical wavelength of 210nm was therefore changed to the less sensitive 280nm for preparative work. For sample collection, a Gilson 204 fraction collector was used. Injections were performed and the relevant peaks collected using an automation facility on the fraction collector, requiring minimal supervision. It was also possible to manually collect fractions without the use of a fraction collector. In a short space of time with only small modifications, the analytical instrumentation was ready for semi-preparative work.

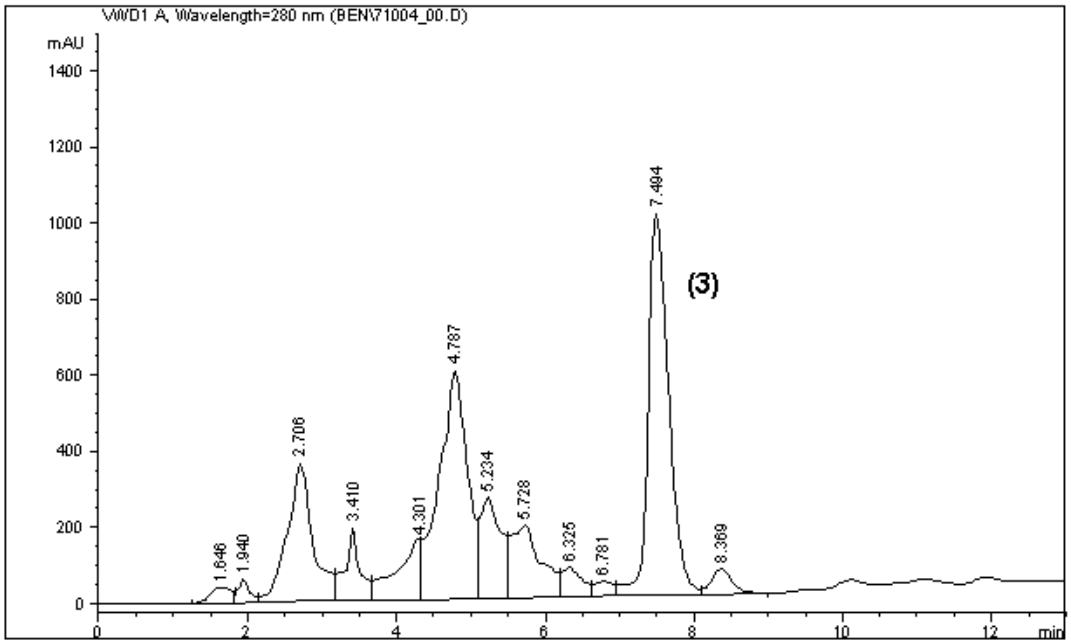


Figure 5. A semi-preparative scale HPLC chromatogram obtained for a mixture containing 3.

Figure 5 shows a typical semi-preparative chromatogram from the original synthesis mixture of **3**. As expected, all retention times are faster than in the analytical run in

Figure 3 and resolution is generally lower due to the higher sample concentrations injected[14]. Ultimately, the recovery yield and percentage purity of the target are the important parameters. In one hour, unattended, 55mg of 97.6% pure **3** was isolated from 120mg of a mixture of no less than ten components (Figure 6). This represents a recovery percentage of about 90%. Given that the observed absorption coefficients for **2** and **3** are similar, this figure was deemed quite accurate.

Alone theoretically, open or flash column chromatography could not match this separation in terms of separation efficiency or time[10]. Practical considerations include the fact that open column or flash chromatography is more prone to operator error, with increased possibility of product loss.

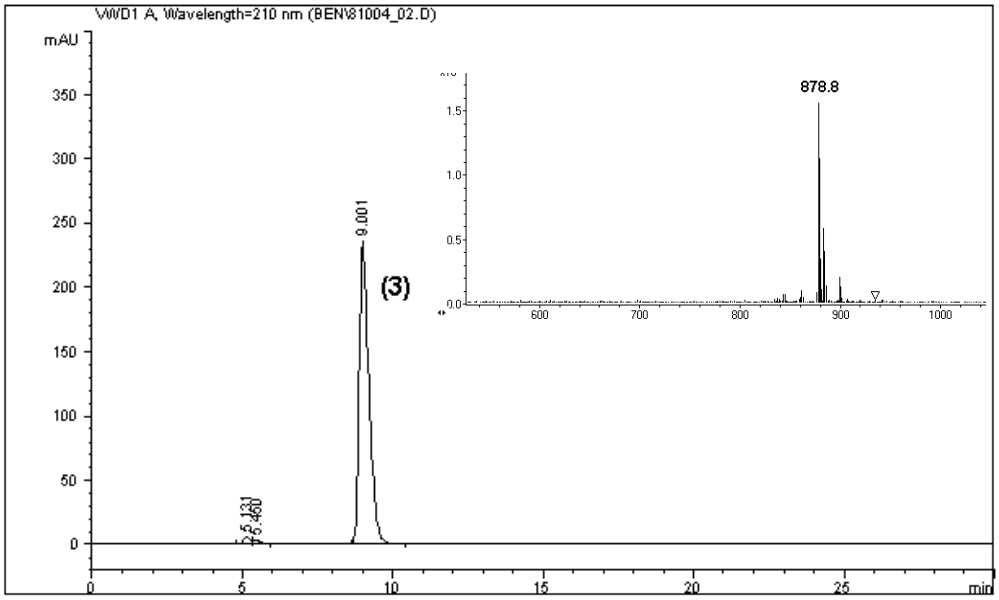


Figure 6. LC-MS chromatogram showing 97.6% pure 3 following semi-preparative HPLC separation. Inset shows MS identification of 3.

Conclusion: Following simple alterations to an analytical LC-MS instrumental setup and synthetic method, in 1 hr, quantities of calixarene **3** were isolated that are sufficient for characterisation, activity screening and for further synthesis. MS also revealed that **3** may be predisposed towards forming complexes with ammonium ions,

rather than the more usual sodium ions. Energy minimised structures suggest that this may be due to the spatial arrangement of the nitrile groups, which are out of plane compared to the well known symmetrically substituted tetraester calix[4]arenes, and form an alternative binding site further away from the phenoxy oxygen atoms at the base of the calix[4]arene annulus. Hence **3** could provide a route to the generation of a range of new calix[4]arene derivatives with dramatically different ion-binding selectivity.

Materials and methods: HPLC was carried out using a HP1100 with UV detection. For MS work, this was coupled to a Bruker/Hewlard-Packard Esquire system, using a positive ESI source and the software's default 'smart' settings. Mobile phase used was isocratic LC grade Acetonitrile with 0.25% formic acid content. This also served as the sample solvent. For analytical LC-MS, a Synergy 150.0 x 2.0mm, 4µm Fusion-RP column was used. Flowrate was 0.2ml/min. Detection wavelength was 210nm. Injections were 5µl of 0.5mg/ml sample. For semi-preparative HPLC, a Synergy 250.0 x 10.0mm, 10µm Fusion-RP column was used. Flowrate was 5.0ml/min. Detection wavelength was 280nm. Injections were 100µl of 300mg/ml sample, filtered before use. Fraction collection was carried out manually or with a Gilson 204 fraction collector in automation mode. Recovery yield was based on % of total peak area.

NaH used was a 60% dispersion in mineral oil. All reactions were carried out under argon. The name *p-tert*-Butylcalix[4]arene was used instead of the IUPAC name for convenience: 5,11,17,23-tetra-*p-tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene.

Potentiometric membranes were prepared using 250mg 2-Nitrophenyl octyl ether, 125mg PVC, 2.5mg **3** and 0.5mg potassium tetrakis(4-chlorophenyl) borate.

The electrochemical cell used consisted of a double junction reference electrode and a PVC membrane working electrode in the following arrangement:

Ag │ AgCl │ 3M NaCl ‖ 0.1M LiOAc ‖ sample solution │ PVC membrane │ 0.1M NH_4Cl │ AgCl │ Ag.

Membranes were conditioned in 0.1M ammonium chloride for 3 hours and followed by deionised water for half an hour prior to analysing the 10^{-1}M cation chloride solution of interest. The potentiometric cell was interfaced to a PC using a National Instruments SCB-68 4-channel interface.

Energy minimised molecular models were generated using Chem3D pro v.8.0 software, also used to calculate extended Huckel charges to display partial charge surfaces.

5,11,17,23-Tetra-*p-tert*-butyl-25,27-bis[(cyanomethyl)-oxy]-26-28-dihydroxycalix[4]arene (2). *p-tert*-Butylcalix[4]arene **1** (5.0g, 7.72mmol), K_2CO_3 (1.28g, 9.26mmol) and bromoacetonitrile (1.95g, 16.20mmol) was heated in CH_3CN (80ml) at 50 ^oC for 5 days. The reaction was monitored by LC-MS. The solvent was evaporated and the residue taken up in CH_2Cl_2 (300ml), washed with 1N HCl (100ml), H_2O (50ml) and brine (50ml) and dried with Mg_2SO_4. CH_2Cl_2 was evaporated and the residue was recrystallised from $CHCl_3$/MeOH yielding a white solid: yield 73%; mp 285-290 ^oC; UV-vis (ACN) 210nm (ε/L cm^{-1} mol^{-1} 152472), 280nm (25974); IR (KBr) 2250 cm^{-1} (CN), 3515 cm^{-1} (OH) ; ^1H NMR δ 7.12 (s, 4H), 6.73 (s, 4 H), 4.81 (s, 4H), 4.23 and 3.45 (ABq, 4H, J = 13.6), 1.33 (s, 18H), 0.87 (s, 18H); ^{13}C NMR δ 150.3 (s), 149.0 (d), 142.9 (d), 135.4 (s), 128.2 (s), 126.6 (s), 125.7 (s), 115.5 (s), 60.8 (s), 34.4 (t), 31.9 (t), 31.5 (s); ESI mass spectrum +m/e 749.6 ([M

+ Na^+], calcd 749.4); HPLC purity: 96.8%. Anal. Calcd for $C_{52}H_{66}N_2O_4$: C, 79.30; H, 8.04; N, 3.85. Found: C, 78.94; H, 7.87; N, 4.00.

5,11,17,23-Tetra-*p-tert*-butyl-25,27-bis[(cyanopropyl)-oxy]-26-28-bis[(cyanomethyl)-oxy]calix[4]arene (3). Calixarene **2** (4.0g, 5.5mmol) and NaH (0.44g, 11.0mmol) was stirred 1 h at room temperature in anhydrous DMF (100ml). 4-Bromobutyronitrile (1.63g, 11.0mmol) was added batch wise and the mixture was stirred at 80 ^oC for 24 h. The reaction was monitored by HPLC-MS. The mixture was cooled and another equivalent of NaH and 4-Bromobutyronitrile was added and heated as before. After a further 72 h the DMF was evaporated and the residue taken up in CH_2Cl_2 (250ml), washed with 1N HCl (100ml), H_2O (50ml), brine (50ml) and saturated NH_4Cl (50ml) and dried with Mg_2SO_4. After filtration the CH_2Cl_2 was removed to give 4.69g of a beige solid. The solid was purified by semi-preparative HPLC to yield a white solid: Recovery yield 90%; mp 234-236 ^oC; UV-vis (ACN) 210nm (ε/L cm^{-1} mol^{-1}150366), 280nm (28420); IR (KBr) 2248cm^{-1} (CN); ^1H NMR δ 7.17 (s, 4H), 6.43 (s, 4 H), 4.95 (s, 4H), 4.33 and 3.28 (ABq, 4H, J = 13.0), 3.90 (t, 4H), 2.69 (t, 4H), 2.32 (m, 4H), 1.35 (s, 18H), 0.80 (s, 18H); ^{13}C NMR δ 152.3 (d), 148.3 (s), 145.8 (s), 135.7 (s), 131.5 (s), 126.7 (s), 125.3 (s), 119.7 (s), 118.0 (s), 74.2 (s), 58.5 (s), 34.7 (s), 34.3 (d), 31.7 (t), 26.3 (s), 15.0 (s); ESI mass spectrum +m/e 878.8 ([M + NH_4^+], calcd 878.6); HPLC purity: 97.6%. Anal. Calcd for $C_{56}H_{68}N_4O_4$: C, 78.10; H, 7.96; N, 6.51. Found: C, 77.79; H, 8.13; N, 6.26.

Acknowledgements: Funding for BS was provided by Enterprise Ireland, grant code SC/2002/161**.** We also thank Science Foundation Ireland for support under the Adaptive Information Cluster award (SFI03/IN3/1361).

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Abbreviated title:

HPLC and MS for preparing asymmetrically substituted calixarenes.

Graphical Abstract:

Isolation and characterisation of an asymmetric calix[4]arene tetranitrile derivative using liquid chromatography and mass spectrometry.

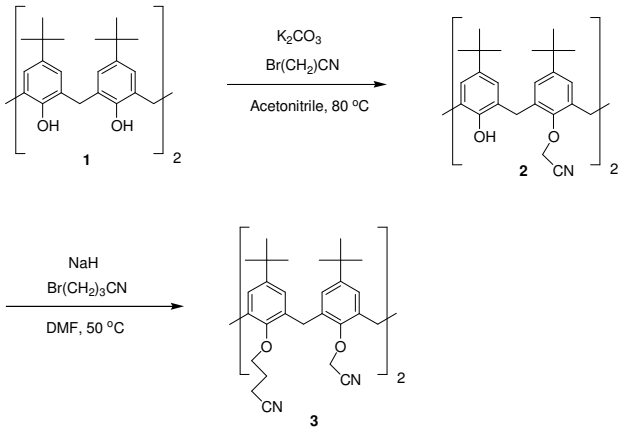
Benjamin Schazmann, Gillian McMahon, Kieran Nolan and Dermot Diamond*.
National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Dublin 9, Ireland.

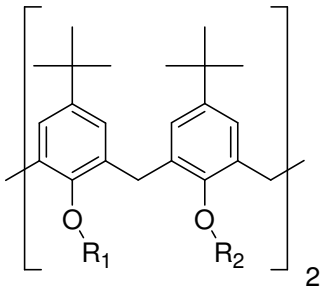
Abstract

A route to the isolation and characterisation of mg quantities of an asymmetric calix[4]arene tetranitrile derivative from a complex reaction mixture using analytical LC-MS (Liquid Chromatography Mass Spectrometry) is described.

Tables, Schemes and Figures:

Scheme 1. Synthesis of 3.





Calixarene	R_1	R_2
1	H	H
2	H	CH_2CN
3	$(CH_2)_3CN$	CH_2CN
4	$(CH_2)_3CN$	$(CH_2)_3CN$
5	$CH_2C(O)_2Et$	$CH_2C(O)_2Et$
6	$CH_2C(O)_2Et$	$C_2H_4C(O)_2Et$

Figure 1. Structural formulae of calix[4]arenes.

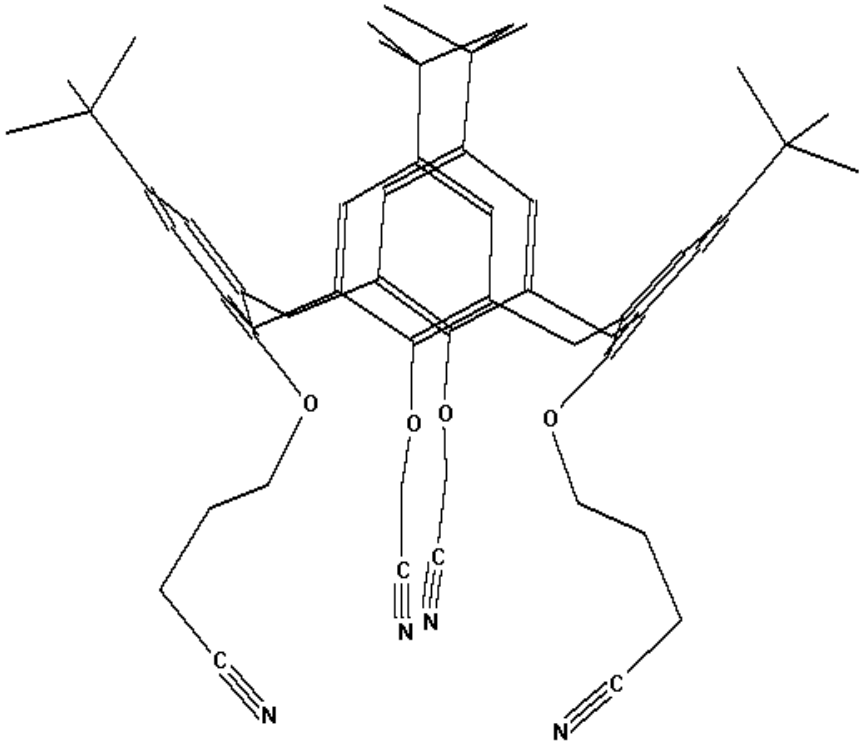


Figure 2. Energy Minimised model of 3 generated using MM2 in Chem 3-d Pro V.8.0 (Cambridgesoft Corporation).

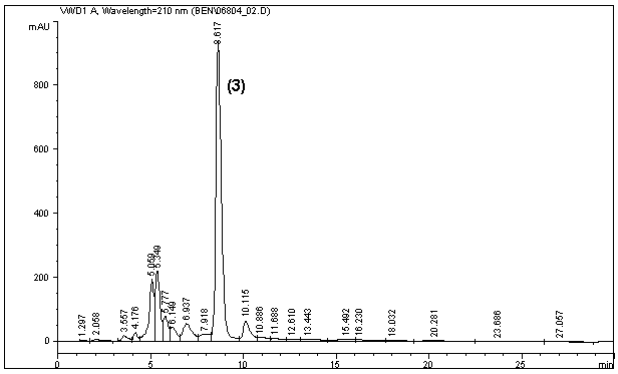


Figure 3. LC chromatogram of reaction mixture; 3 identified by MS.

Cation	Potential change (mV)
K^+	+216.0
NH_4^+	+206.9
Na^+	+154.0
Ca^{2+}	+79.4
Li^+	+71.4
Mg^{2+}	+62.4

Table 1. Potential changes of an ISE containing 3 when analysing 10^{-1}M aqueous solutions of the indicated cation chloride.

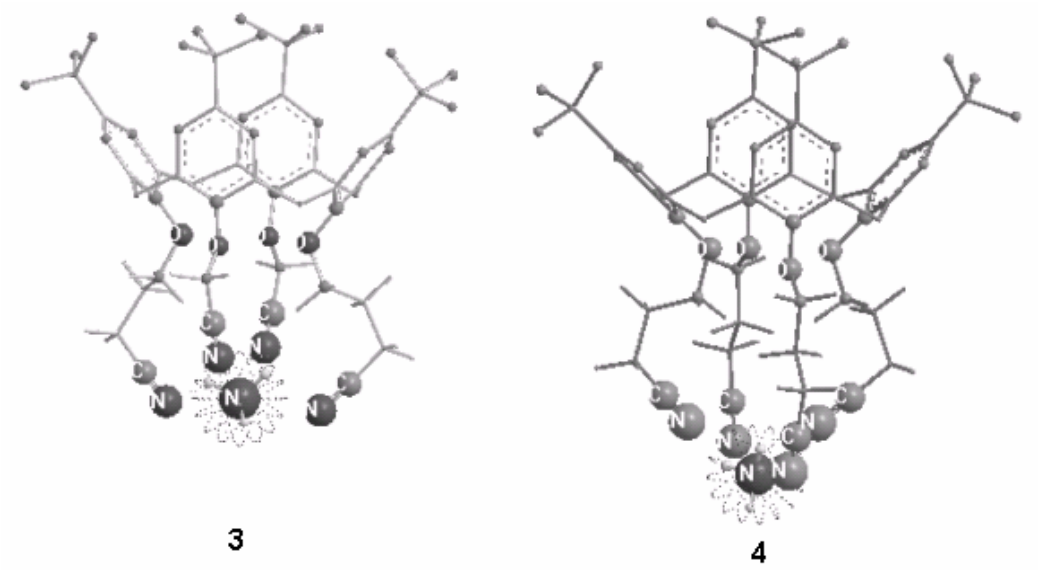


Figure 4. Complexes of 3 and 4 with an ammonium ion. Atoms are scaled by size according to Huckel partial charges, revealing the importance of the nitrile groups for complexation.

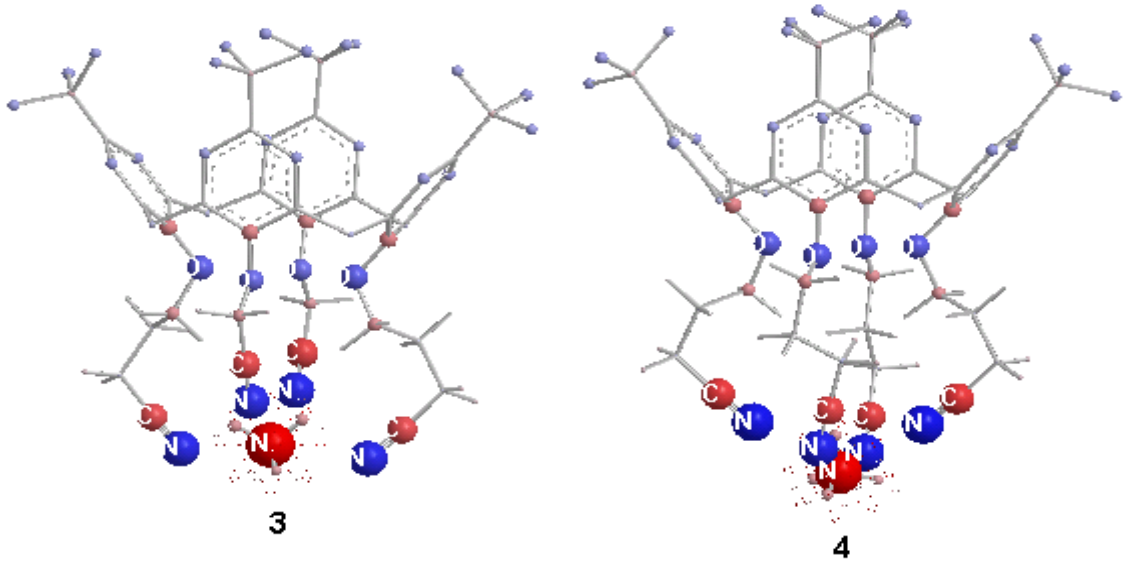


Figure 4. Complexes of 3 and 4 with an ammonium ion. Atoms are scaled by size according to Huckel partial charges. Red and blue are areas of positive and negative localised charge respectively. The importance of the nitrile groups for complexation is revealed.

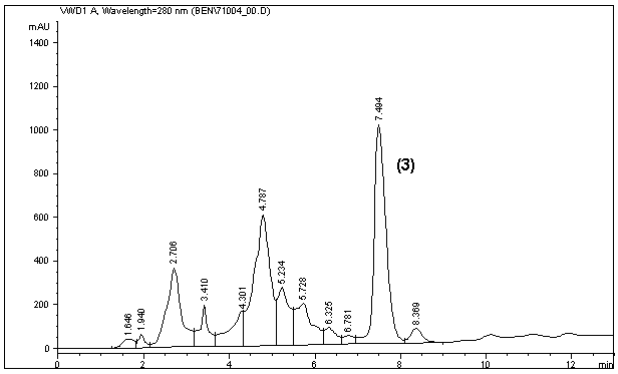


Figure 5. A semi-preparative scale HPLC chromatogram obtained for a mixture containing 3.

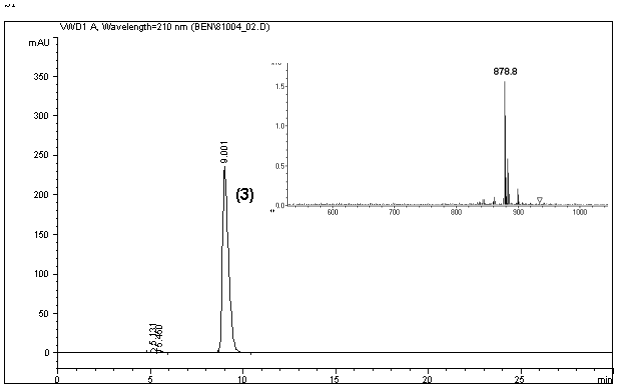


Figure 6. LC-MS chromatogram showing 97.6% pure 3 following semi-preparative HPLC separation. Inset shows MS identification of 3.