PAPER

NJC
www.rsc.org/njc

Multiple ionic interactions for noncovalent synthesis of molecular capsules in polar solvents

Francesca Corbellini,[a] Fijs W. B. van Leeuwen,[a] Hans Beijleveld,[a] Huub Kooijman,[b] Anthony L. Spek,[b] Willem Verboom,[a] Mercedes Crego-Calama*[a] and David N. Reinhoudt*[a]

[a] *Laboratory of Supramolecular Chemistry and Technology, MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands. E-mail: m.cregocalama@utwente.nl; Fax: +31 53 489 4645; Tel: +31 53 4892984*

[b] *Department of Crystal and Structural Chemistry, Utrecht University, H. R. Kruytgebouw, N-827 Padualaan 8, 3584 CH Utrecht, The Netherlands. E-mail: h.kooijman@chem.uu.nl; Fax: +31 30 2533940; Tel: +31 30 2532533*

Received (in Montpellier, France) 11th August 2004, Accepted 18th October 2004
First published as an Advance Article on the web 8th December 2004

The formation and characterization of molecular capsules resulting from the self-assembly between oppositely charged (thia)calix[4]arenes in polar solvents like MeOH and MeOH–H_2O are reported. The multiple ionic interactions allow the self-assembly of the complementary (thia)calix[4]arenes **1** and **2a–d** into 1 : 1 complexes as revealed by ^{1}H NMR and mass spectrometry (ESI-MS). Isothermal titration calorimetry (ITC) was used to determine the association constants, which, depending on the ionic groups involved in the complexes, vary between 10^3 and 10^6 M^{-1}. An X-ray structure of the assembly **1•2d** was also obtained. Unlike in solution, in the solid state **1•2d** forms a 1 : 1 three-dimensional network in which **2d** adopts a 1,2-*alternate* conformation.

Introduction

Building molecular structures that are able of encapsulating guest molecules in solution is one of the main objectives in supramolecular chemistry.[1] Synthetic capsules and cages of different sizes and shapes have been obtained from different subunits specifically functionalized to assemble, *via* noncovalent interactions, into well-defined architectures.[2] So far the predominant interactions employed in the synthesis of molecular capsules have been hydrogen bonds[2b,3] and metal–ligand interactions.[4] Recently, molecular capsules have also been obtained by combining different noncovalent interactions.[5] The resulting three-dimensional cavity-containing hosts have found applications in sensing,[6] catalysis,[7] selective recognition and molecular storage.[8] Nevertheless, among others, one of the goals of supramolecular chemistry is to create macromolecular capsules for biochemical applications such as drug encapsulation, transport through cell membranes and drug delivery. To achieve these objectives, it becomes obvious that efforts have to be focused on developing systems that form and display stable association as well as binding properties in aqueous solution.

Ionic interactions are often employed as an important attractive force in both biological[9] and artificial molecular recognition.[10] Nevertheless, these strong interactions have been only marginally used for building molecular capsules. Surprisingly, before our initial studies only a few examples of stable cage-like complexes using multiple ionic interactions have been reported in the literature and only very recently a few other publications have followed these first examples.[11]

Previously, we proved the effectiveness of multiple ionic interactions as an alternative to hydrogen bonds and metal–ligand coordination for the synthesis of noncovalent supramolecular capsules in aqueous solutions. Calix[4]arenes functionalized at their upper rim with oppositely charged groups form molecular capsules in polar organic solvent where guest encapsulation can be observed.[12] In parallel with our study, the synthesis of molecular capsules based on ionic interactions between oppositely charged calix[4]- and calix[6]-arenes has been published by the group of Schrader.[13]

Here, a more extensive study is presented on the use of ionic interactions for the synthesis of supramolecular capsules in polar solvents. We report that strong association between the oppositely charged calix[4]arenes **1** and **2a–c**, functionalized at the upper rim with amidinium and either sulfonate, carboxylic acid and phosphonate moieties, respectively, results in the formation of 1 : 1 molecular capsules (Chart 1). Capsule formation between **1** and the larger and less preorganized thiacalix[4]arene **2d** has also been investigated. The four sulfur atoms in the thiacalix[4]arene **2d** provide an enlarged skeleton compared to the calix[4]arene scaffold.[14] As suggested by molecular

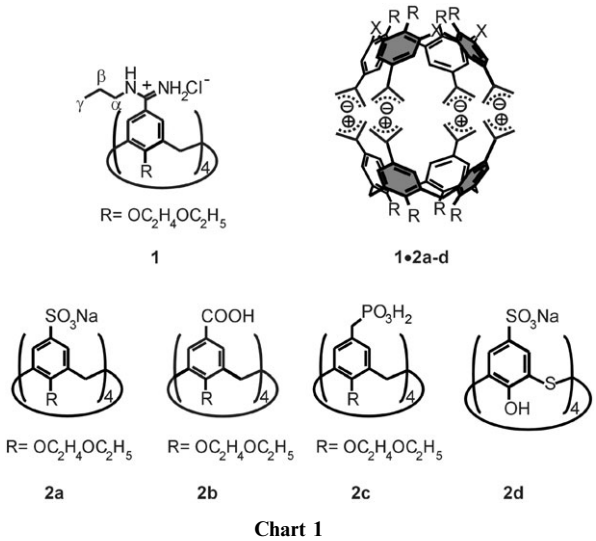


Chart 1

DOI: 10.1039/b412409j

modelling studies, the resulting capsule **1•2d** is suitable for the encapsulation of larger guest molecules.

Experimental

General information and instrumentation

The reagents used were purchased from Aldrich or Acros Chimica and used without further purification. All the reactions were performed under a nitrogen atmosphere. Analytical thin layer chromatography was performed using Merck 60 F_{254} silica gel plates. Column chromatography was carried out on Merck silica gel 60 (230–400 mesh) and reverse phase chromatography on Silica RP-18. Ion exchange chromatography was carried out on DOWEX 1-X8, 50–100 mesh, Cl form. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA (300 MHz) or a Varian Unity 400 WB NMR spectrometer. ^{1}H NMR chemical shift values (300 MHz) are reported as δ using the residual solvent signal as an internal standard (CHD_2OD, $\delta = 3.30$; DMSO-d_6, $\delta = 2.49$; $CDCl_3$, $\delta = 7.29$). ^{13}C NMR chemical shift values (100 MHz) are reported as δ using the residual solvent signal as an internal standard (CD_3OD, $\delta = 49.0$; DMSO-d_6, $\delta = 39.7$; $CDCl_3$, $\delta = 77.0$). Infrared spectra were recorded on a FT-IR Perkin Elmer Spectrum BX spectrometer and only characteristic absorptions are reported. Fast atom bombardment (FAB) mass spectra were recorded with a Finnigan MAT 90 spectrometer. Electrospray ionization (ESI) mass spectra were recorded on a Micromass LCT time-of-flight (TOF) mass spectrometer. Samples were introduced using a nanospray source. Matrix-assisted laser desorption ionization (MALDI) TOF mass spectra were recorded using a Perkin Elmer/PerSpective Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer. Elemental analyses were carried out using a 1106 Carlo-Erba Strumentazione element analyzer. Compounds **2a**,[15] **2d**,[16] **4**[17] and **5**[18] were synthesized according to literature procedures. Compound **3**[19] was obtained in 78% yield following a procedure reported for the analogous 5,11,17,23-tetracyano-25,26,27,28-tetrapropoxycalix[4]arene.[20]

Binding studies

The titration experiments were carried out using a Microcal VP-ITC microcalorimeter with a cell volume of 1.4115 ml. The formation of the assemblies **1•2a–d** was studied by adding aliquots of a 1 mM solution of **1** to a 0.1 mM solution of **2a–d**, in the calorimetric cell, and monitoring the heat change after each addition. Dilution effects were determined in a second experiment by adding the solution of **1** into the solvent mixture and subtracting this contribution from the raw titration. The final curves were modelled using a 1 : 1 nonlinear regression analysis. The fittings were done using Microcal Origin® software. Borate buffer was prepared by dissolving $Na_2B_4O_7 \cdot 10\ H_2O$ in MeOH–H_2O ($x_{water} = 0.4$).

Syntheses

5,11,17,23-Tetrakis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (6). This compound was synthesized by a procedure reported for the analogous 5,11,17,23-tetrakis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy) calix[4]arene (1,3-*alternate*).[21] Compared to the reported procedure, a longer reaction time was required to obtain the tetrasubstituted compound **6**. Shorter reaction times lead to mixtures of partially substituted derivatives. To a suspension of **5** (600 mg, 0.84 mmol) and paraformaldehyde (0.40 mg, 4.45 mmol) in dioxane (45 ml), acetic acid (1.92 ml, 33.66 mmol), phosphoric acid (4.06 ml, 67.3 mmol) and concentrated HCl (5.7 ml) were added. The reaction mixture was heated at 80 °C for 3 days. The reaction was monitored by TLC (silica, hexane–ethyl acetate 8 : 2). After cooling the mixture was poured into ice water and extracted with CH_2Cl_2 (3 × 100 ml). The organic layer was washed with water (3 × 200 ml), brine (3 × 200 ml) and dried over $MgSO_4$. The solution was evaporated to dryness and the residue recrystallized from dichloromethane–hexane (1 : 2). Yield: 45%. For the spectral data see ref. 22.

5,11,17,23-*N*-Propyltetraamidinium-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene, tetrachloride salt (1). To a solution of Me_2AlCl (1.0 M in hexane; 20.6 ml, 20.6 mmol) at 0 °C, 1-propylamine (1.69 ml, 20.6 mmol) in fluorobenzene (5 ml) was added dropwise. The mixture was stirred for 1 h at 25 °C and then a solution of compound **3** (700 mg, 0.86 mmol) in fluorobenzene (5 ml) was added and the solution left under stirring for 1 h at room temperature and then at 80 °C for 5 days. The reaction was monitored by TLC (silica, *n*-BuOH–CH_3COOH–H_2O 3 : 1 : 1). The mixture was cooled to 0 °C and quenched with ice and MeOH and the suspension filtered through a pad of Hyflo Super Cel®. The solid was washed with MeOH and water and the filtrates were treated with an excess of 3 N HCl and then concentrated in vacuum. The solid residue was submitted to preparative reverse phase chromatography using a step gradient of EtOH–H_2O up to 40% (v/v) as the eluent. Ion exchange column chromatography using H_2O as the eluent gave the corresponding chloride salt of **1** as a white solid in 65% yield. M.p. > 215 °C (dec.). ^{1}H NMR (CD_3OD): $\delta = 7.36$ (s, 8H), 4.77 (d, 4H, $J = 14.1$), 4.33 (t, 8H, $J = 6.0$), 3.88 (t, 8H, $J = 6.0$), 3.53 (q, 8H, $J = 6.9$), 3.45 (d, 4H, $J = 13.8$), 3.33 (t, 8H, $J = 7.2$), 1.72 (m, 8H), 1.17 (t, 8H, $J = 7.2$), 1.01 (t, 12H, $J = 7.2$). ^{13}C NMR (CD_3OD): $\delta = 162.43$, 160.41, 135.05, 127.95, 122.38, 73.60, 68.95, 65.44, 43.60, 30.03, 20.21, 13.72, 9.63. IR (KBr): $\nu =$ 2965, 1668, 1623, 1467, 1383, 1224, 998 cm^{-1}. MS (FAB): m/z 1049.7 $[M - 4HCl + H]^+$ (calcd 1049.67). Anal. calcd for $C_{60}H_{92}Cl_4N_8O_8 \cdot H_2O$: C 59.40, H 7.81, N 9.24; found: C 59.27, H 7.76, N 9.13.

5,11,17,23-Tetracarboxy-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (2b). *tert*-Butyllithium (1.7 M in pentane; 11.05 ml, 18.8 mmol) was added to a solution of **4** (2 g, 2.07 mmol) in dry THF (50 ml) under nitrogen at −78 °C. The reaction mixture was stirred at −78 °C for 45 min. After removal of the cooling bath, CO_2 gas, dried in conc. H_2SO_4, was bubbled through the solution for 1 h. The reaction mixture was treated with 6 M HCl (20 ml). The solution was evaporated to dryness, the solid was dissolved in ethanol (400 ml) and the solution refluxed for 15 min. On gradually cooling down to room temperature a white precipitate appeared, which was filtered off and dried *in vacuo* over P_2O_5. Yield 72%. M.p. > 250 °C. ^{1}H NMR (DMSO-d_6): $\delta = 12.34$ (s, 4H), 7.32 (s, 8H), 4.32 (d, 4H, $J = 8.8$), 3.91 (t, 8H, $J = 4.9$), 3.37 (d, 4H, $J = 9.0$), 1.87 (m, 8H), 1.42 (m, 8H), 0.97 (t, 12H, $J = 7.3$). ^{13}C NMR (DMSO-d_6): $\delta = 167.13$, 160.21, 134.97, 130.01, 124.97, 75.11, 32.11, 30.37, 19.14, 14.18. MS (FAB): m/z 886.8 $[M - H]^-$ (calcd 887.3). Anal. calcd for $C_{48}H_{56}O_{12}$: C 69.88, H 6.84; found C 70.13, H 6.83.

5,11,17,23-Tetrakis(phosphonomethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (2c). Compound **6** (200 mg, 0.22 mmol) was refluxed in triethylphosphite (3 ml) for 24 h. After cooling, unreacted triethylphosphite was distilled under reduced pressure and the residue dried under vacuum. Bromotrimethylsilane (TMSBr; 0.78 ml, 5.97 mmol) was added and the solution stirred overnight at room temperature. TMSBr was removed under vacuum. Methanol (10 ml) was added and the solvent was removed under reduced pressure. Addition of water gave a white precipitate that was filtered, washed with water and dried over P_2O_5. Yield: 54%. M.p. > 250 °C. ^{1}H NMR (CD_3OD): δ 6.65 (s, 8H), 4.49 (d, 4H, $J = 13.2$), 4.07 (t, 8H, $J = 5.4$), 3.88 (t, 8H, $J = 5.4$), 3.57 (q, 8H, $J = 6.9$), 3.10 (d, 4H, $J = 13.2$), 2.80 (d, 2H, $J = 21.3$) 1.21 (t, 12H, $J = 6.9$). ^{13}C NMR (CD_3OD): $\delta =$ 166.53, 162.80, 137.09, 129.80, 122.60, 75.44, 70.72, 67.17,

31.68, 15.39. IR (KBr): ν = 3366, 1471, 1056, 976 cm^{-1}. MS (FAB): m/z = 1088.32 $[M + H]^+$ (calcd 1088.67).

Crystal structure determination

Diffraction data for **1•2d** were collected at T = 150 K with graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å) on a Nonius KappaCCD diffractometer with a rotating anode for $1.00° < \theta < 25.25°$; no absorption correction was applied. The structure was solved by automated direct methods (SHELXS86)[23] and refined on F^2 using full-matrix least-squares techniques (SHELXL97).[24] Electron density in a disordered solvent area located at a crystallographic inversion center was taken into account in the refinement using PLATON/SQUEEZE.[25] Where relevant, data cited below are given without disordered solvent contribution. Three ethoxyethoxy groups of **1** were refined with a two-site disorder model. The water hydrogen atoms were placed at calculated positions corresponding to ideal H-bond geometry. All ordered non-hydrogen atoms were refined with anisotropic displacement parameters; the isotropic displacement parameters of hydrogen atoms were linked to the equivalent isotropic displacement parameter of their carrier atoms. Refinement of 1379 parameters converged at a final $wR2$ value of 0.2676, $R1$ = 0.0995 [for 17719 reflections with $I > 2\sigma(I)$], S = 1.282, −1.30 e $Å^{-3} < \Delta\rho <$ 2.28 e $Å^{-3}$ (near disordered ethoxyethoxy groups).

Crystal data for **1•2d**: $C_{60}H_{92}N_8O_8 \cdot C_{24}H_{12}O_{16}S_8 \cdot 8C_2H_6OS \cdot 0.776H_2O$, M_r = 2505.39, colorless, block-shaped crystal (0.1 × 0.2 × 0.3 mm), triclinic, space group $P\bar{1}$ (no. 2) with a = 18.061(2), b = 20.378(2), c = 25.193(4) Å, α = 69.128(15)°, β = 69.681(15)°, γ = 69.840(18)°, U = 7862.2(17) $Å^3$, Z = 2, D_c = 1.058 g cm^{-3}, $F(000)$ = 2656, μ(Mo Kα) = 0.279 mm^{-1}, 155874 reflections measured, 28453 independent, R_{int} = 0.0831.†

Result and discussion

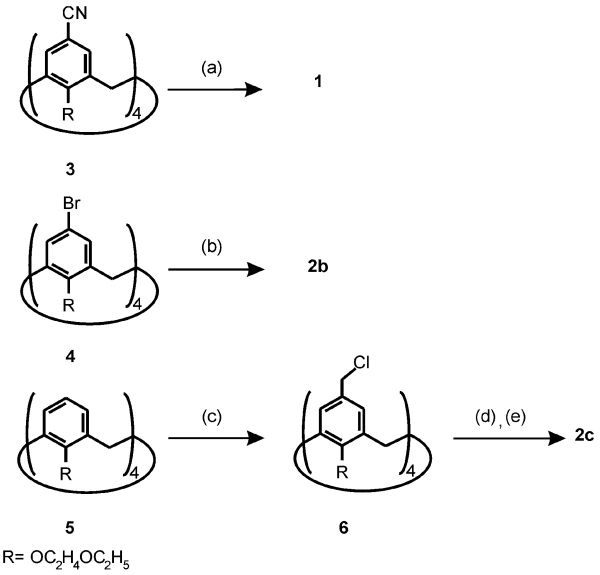
Synthesis

Calix[4]arenes fixed in the *cone* conformation by *O*-alkylation at the lower rim offer a preorganized cavity that is easily functionalized at the upper rim.[26] Calix[4]arene **1**, with tetra-amidinium groups directly attached to the upper rim, was synthesized using a modified one-step literature procedure.[27] The introduction of the amidinium moieties was achieved by reacting the alkylchloroaluminium amide, generated from Et_2AlCl and 1-propylamine, with cyanocalix[4]arene **3** in fluorobenzene (Scheme 1). The chloride salt of **1** was obtained after reverse phase chromatography and ion exchange chromatography in 65% yield. Calix[4]arene **2a**[15] and thiacalix[4]arene **2d**[16] were synthesized according to literature procedures. Tetracarboxycalix[4]arene **2b** was obtained in 72% yield in a Br/Li exchange reaction of the precursor compound **4** with *t*-BuLi in THF at −78 °C, followed by treatment with CO_2 (Scheme 1).

The synthesis of compound **6** was carried out in an alternative way to the ones reported in the literature,[18,22] following a similar route as described for the synthesis of the analogous 5,11,17,23-tetrakis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)-calix[4]arene (1,3-*alternate*).[21]

Reaction of the chloromethylated calix[4]arene **6** with triethylphosphite, under Arbuzov conditions, and subsequent hydrolysis with TMSBr afforded calix[4]arene **2c** in 54% yield (Scheme 1).

Compounds **1** and **2a–c** give sharp ^1H NMR spectra in CD_3OD, showing that they do not aggregate in solution. Moreover, the presence of one pair of doublets for the bridge methylene protons clearly reveals the C_{4v} symmetry common to tetrasubstituted *cone* calix[4]arenes. Unlike the analogous



Scheme 1 Reagents and conditions: (a) Et_2AlCl, 1-propylamine, fluorobenzene, 5 days, 80 °C, yield 65%; (b) *t*-BuLi, CO_2, 30 min, rt, HCl 6M, yield 72%; (c) *p*-HCOH, HCl, CH_3COOH, H_3PO_4, 1,4-dioxane, 3 days, reflux, 45%; (d) $(EtO)_3P$, 24 h reflux; (e) TMSBr, 12 h rt, then MeOH, yield 54%.

tetrasulfonatecalix[4]arene **2a**, which is fixed in the *cone* conformation by the alkylation of the phenolic oxygens, tetrasulfonatethiacalix[4]arene **2d** can exist in solution in four different conformations. However, according to density functional theory calculations of the structure and conformational equilibrium of thiacalix[4]arene, the most stable conformer is the *cone*, which is stabilized by a cyclic array of hydrogen bonds at the lower rim.[28] The presence of only a single peak (~8 ppm) for the aromatic protons in the ^1H NMR spectrum of the thiacalix[4]arene **2d** in CD_3OD indicates indeed the *cone* conformation.

Characterization of the molecular capsules 1•2a–d

Isolation of the 1 : 1 complexes **1•2a–d** was achieved by precipitation in water. While all the isolated building blocks are soluble in water, the mixtures of the two corresponding components were insoluble at room temperature. The white precipitates were filtered, washed with water, dried and redissolved in CD_3OD. Integration of the ^1H NMR resonances revealed in each case the 1 : 1 (or n : n) stoichiometry of the complexes.

Interestingly, the proton signals of the amidinium propyl chains of compound **1**, H_α, H_β and H_γ, shifted upfield upon assembly formation (Fig. 1 and Table 1). In analogy to what was observed before,[12,29] this upfield shift is attributed to the inclusion of the propyl side chain of calix[4]arene **1** in the cavity of the formed capsule **1•2**.

For assembly **1•2a** only small changes were observed for all the other signals ($\Delta\delta < 0.1$ ppm), while for the assembly **1•2b** downfield changes were observed also for the methylene bridge protons ($\Delta\delta$ = 0.12 ppm). Downfield shifts accompanied by broadening were also detected for the first CH_2 group of the ethoxyethyl chains at the lower rim of **2b** ($\Delta\delta$ = 0.15 ppm). Assembly **1•2c** shows only minor changes in the resonances of the propyl side chain protons (Table 1). Most likely the hindrance caused by the bulkier phosphonate groups precludes the access of the propyl chain to the cavity of the calix[4]arene **1**. Nevertheless, small changes are detectable for the signals of the aromatic protons and the methylene bridge hydrogens of **2c** ($\Delta\delta \sim 0.05$ ppm). Analogously to what is observed for the assemblies based on calix[4]arenes, upfield shifts in the resonances of the protons of the amidinium chains of **1** (Table 1)

† CCDC reference number 253844. See http://www.rsc.org/suppdata/nj/b4/b412409j/ for crystallographic data in .cif or other electronic format.

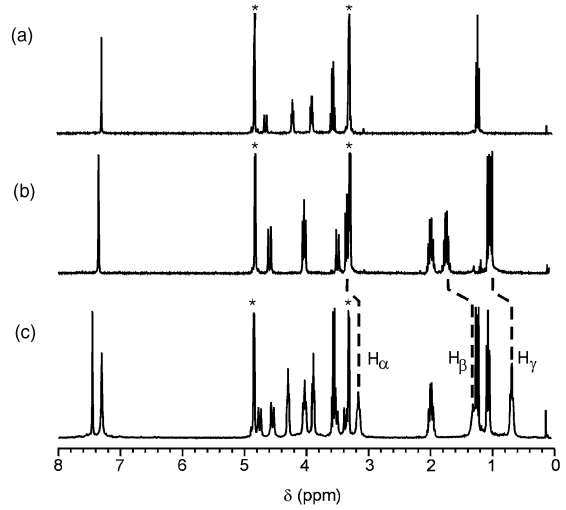


Fig. 1 ^{1}H NMR spectra (CD_3OD, 298 K) of (a) **2a**, (b) **1** and (c) capsule **1•2a** obtained by precipitation in H_2O upon mixing solutions of **1** and **2a**. (*) indicates solvent signals. (For proton labelling see Chart 1.)

and of the aromatic protons of both building blocks [$\Delta\delta$ (**1**) = 0.23 ppm, $\Delta\delta$ (**2d**) = 0.11 ppm] were observed for assembly **1•2d**. This is a good indication that a molecular capsule is formed where one of the alkyl chains of **1** is pointing inside the internal cavity.

Crystallization of assembly **1•2d** in DMSO–MeOH provided single crystals suitable for X-ray diffraction studies. In the solid state **1** and **2d** form an infinite three-dimensional network. The unit cell contains, besides **1** and **2d** in a 1 : 1 ratio, eight molecules of DMSO, one molecule of water and a number of completely disordered solvent molecules, which are located in a region around the crystallographic inversion centre. Thiacalix[4]arene **2d** adopts a 1,2-*alternate* conformation, thus presenting two adjacent sulfonate moieties on each side of the plane. Calix[4]arene **1** adopts instead a *pinched-cone* conformation in which the two opposite aromatic rings are bent inwards and the other two aromatic rings are forced to bend outwards. A very complicated structure is formed in which hydrogen bonds are formed either between the two building blocks or between one of the building blocks and solvent molecules. Fig. 2(a) depicts one type of interaction where two opposite amidinium groups of **1** form hydrogen bonds to one of the sulfonate groups of the thiacalix[4]arene **2d**. Fig. 2(b) shows hydrogen bonds between two adjacent amidinium groups of **1** and two sulfonate moieties of **2d**. Interestingly, the 1,2-*alternate* conformation in **2d** is stabilized by bifurcated hydrogen bonds, involving an intramolecular hydrogen bond between the hydroxyl group and a sulfur atom of **2d** as well as an intermolecular bond donated to the oxygen of a DMSO molecule (not shown).

This unexpected result prompted us to further investigate the assembly of **1•2d** in solution. According to the X-ray structure, compound **2d** in **1•2d** adopts a 1,2-*alternate* conformation. If the same conformation exists in solution, two signals for the

Table 1 ^{1}H NMR chemical shift changes for the protons of the propyl chain of **1** upon formation of the complexes **1•2a–d**, (T = 298 K, CD_3OD). (For assignment of H_α, H_β and H_γ see Chart 1.)

Assembly	$\Delta\delta_{H\alpha}$	$\Delta\delta_{H\beta}$	$\Delta\delta_{H\gamma}$
1•2a	0.23	0.53	0.33
1•2b	0.13	0.16	0.25
1•2c	—	0.03	0.04
1•2d	0.46	0.45	0.32
1•2d[a]	0.27	0.35	0.30

[a] DMSO-d_6.

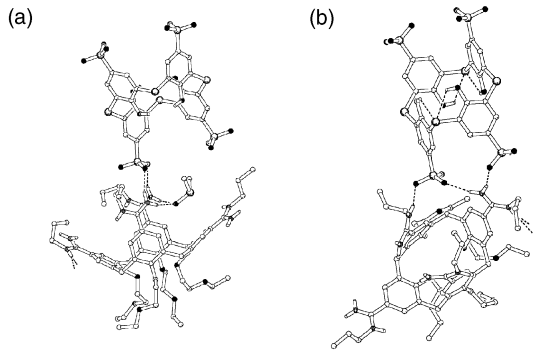


Fig. 2 Portions of the X-ray structure of the 3D network showing the hydrogen-bonding interactions between calixarene **1** and thiacalix[4]-arene **2d**. See text for details of views (a) and (b).

aromatic protons of **2d** would be expected, while, for symmetry reasons, one signal would be expected for the *cone* conformation. As mentioned before, in CD_3OD solution at 25 °C, the ^{1}H NMR spectrum of **1•2d** shows a single resonance for the aromatic protons of **2d**, which accounts for a symmetrical conformer (*cone*). However, the singlet could also be an averaged signal of different conformers that are rapidly interconverting. Temperature-dependent ^{1}H NMR spectra of **1•2d** were recorded in CD_3OD. At −50 °C a singlet for the aromatic protons was still observed while a further decrease in the temperature resulted in the precipitation of the thiacalix[4]arene **2d**. This result indicates that if different conformers are present at 25 °C, at −50 °C the flexibility of **2d** is still too high for the individual conformers to be revealed by NMR spectroscopy, which is not very likely, thus confirming the *cone* conformation.

To study the effect of the concentration on the self-assembly of **1** and **2d**, ^{1}H NMR studies in DMSO-d_6, in which a better solubility of the complex is observed, were performed. The ^{1}H NMR spectrum of a 20 mM solution of **1•2d** in DMSO-d_6 showed no shifts in the propyl chains of the amidinium groups and only small upfield changes for the –NH signals of **1** (Fig. 3).

At a much lower concentration (1 mM), a DMSO-d_6 solution of **1•2d** showed a different spectrum, which is analogous to the one observed in CD_3OD (spectra not shown). The signals of the protons of the propyl side chain of **1** were all upfield-shifted (Table 1). Furthermore, significant upfield shifts were also observed for the –NH signals of **1** (upfield shifts, $\Delta\delta$ = 0.62, 0.64, 0.30 ppm). These results show that the concentration somehow affects the geometry of the assembly. The structure obtained in the solid state could exist in concentrated solutions of the two building blocks, while at lower concentration (1 mM) a well-defined complex is most probably formed. This assumption is corroborated by analysis of the assembly **1•2d** by 2D NMR where NOE connectivities between the aromatic protons of **2d** and the protons of the propyl amidinium chain of **1** were found. Perhaps in the solid state the molecules organize by optimizing both space and intramolecular interactions (Fig. 2), while in solution the flexible thiacalix[4]arene **2d** assembles to give a well-defined **1•2d** molecular capsule.

Equimolar solutions of the different assemblies were analyzed by electrospray mass spectrometry (ESI-MS) and in all cases the presence of a doubly charged peak for the capsule was observed. Peaks for the isolated building blocks were also detected. The spectrum of an equimolar solution of **1** and **2a** in MeOH showed the doubly charged signals of the capsule at m/z 1064.8 corresponding to $[(\mathbf{1\bullet 2a}) + 2Na]^{2+}$. A peak at m/z 717.5 for $[(\mathbf{1\bullet 2a}) + 3Na]^{3+}$ was also present. The spectrum of an equimolar solution of **1** and **2b** in MeOH showed the doubly charged signals of the capsule at m/z 969.7 and m/z 987.8 corresponding to $[(\mathbf{1\bullet 2b}) + 2H]^{2+}$ and $[(\mathbf{1\bullet 2b}) + H + Na]^{2+}$, respectively, together with a peak at m/z 1049.5 for [**1** − 4HCl

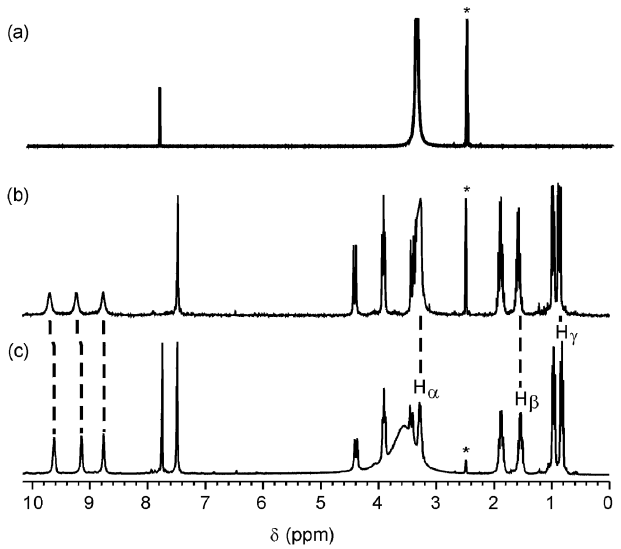


Fig. 3 ^{1}H NMR spectra (DMSO-d_6, 298 K) of (a) **2d**, (b) **1** and (c) **1•2d** (c = 20 mM). (*) indicates solvent signals. (For proton labelling see Chart 1.)

+ H]$^+$ and two peaks at 911.2 and 933.8 for [**2b** + Na]$^+$ and [(**2b** − H + Na) + H]$^+$, respectively (Fig. 4).

Analogously, the measured ESI-MS spectrum of an equimolar solution of **1** and **2c** in MeOH showed the [(**1•2c**) + Na + H]$^{2+}$ and [(**1•2c**) + 2Na]$^{2+}$ ions at m/z 1079.5 and m/z 1090.5 together with signals at m/z 1049 and 911.3 for [**1** − 4HCl + H]$^+$ and [**2c** + Na]$^+$, respectively. For assembly **1•2d** analyses at different concentrations and using different mass spectrometric techniques were performed. A 0.5 mM solution of **1•2d** in MeOH was analyzed by ESI-MS. The spectrum showed a peak at m/z 955.4 corresponding to [(**1•2d**) + 2Na]$^{2+}$. The FAB-MS spectrum showed the presence of a peak at m/z 1865.9 corresponding to [(**1•2d**) + H]$^+$. The same results were obtained from the ESI-MS and FAB-MS of both a 1 mM and 20 mM solution of **1•2d** in DMSO. No evidence for high m/z aggregates was observed. In agreement with the results obtained using ESI-MS and FAB-MS, the MALDI-TOF spectrum of a 20 mM solution of **1•2d** in DMSO showed only the presence of the expected 1 : 1 complex.

Isothermal titration calorimetry (ITC) was used to study the thermodynamics of the association between **1** and **2a–d**. The titrations were carried out by adding aliquots of calix[4]arene tetraamidinium **1** to a solution of calix[4]arenes **2a–d** at 298 K in MeOH–H_2O (x_{water} = 0.4) in the presence of either tetrabutylammonium perchlorate (Bu_4NClO_4, I = 0.01 M) or borate buffer ($Na_2B_4O_7$, I = 0.01 M and I = 0.03 M).

The titration curves showed an inflection point after addition of 1.0 equiv. of **1**, which confirms the formation of complexes with a 1 : 1 stoichiometry (Fig. 5). The titration data were fitted to a 1 : 1 binding model using a nonlinear least-squares fitting procedure. The corresponding association constants and thermodynamic parameters are listed in Table 2.

In the presence of 1.0 × 10^{-2} M (I = 0.01 M) of Bu_4NClO_4 as background electrolyte, association constants in the order of

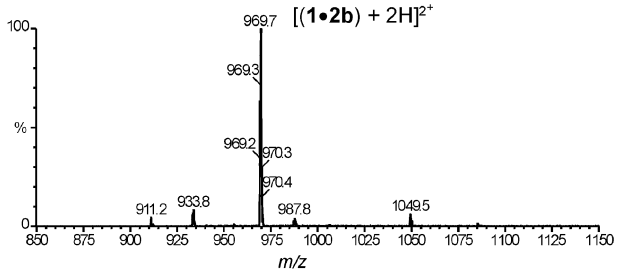


Fig. 4 ESI-MS spectrum for assembly of **1•2b**.

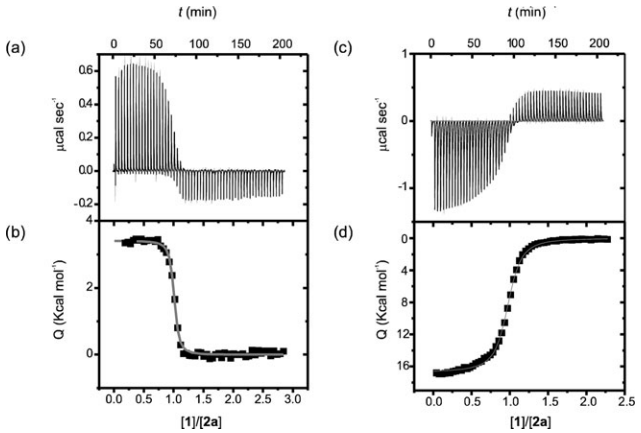


Fig. 5 Calorimetric titration of **2a** (0.1 mM) with **1** (1.0 mM) in MeOH–H_2O (x_{water} = 0.4) at 298 K in the presence of Bu_4NClO_4, I = 0.01 M (left), and $Na_2B_4O_7$, I = 0.01 M, pH = 9.2 (right). (a) and (c) show the heat evolution per injection of **1**; (b) and (d) are the resulting binding curve and best fit curve.

10^6–10^7 M^{-1} for **1•2a** and **1•2b** were determined. The association process is strongly entropy driven, most likely due to the release of highly ordered solvent molecules into the bulk solvent. The positive values obtained for the enthalpies are ascribed to the energy needed to desolvate the charged groups, which overrides the negative enthalpic contribution due to the formation of the ionic interactions of the assemblies.[30] The binding curve obtained for assembly **1•2c** displayed only a very small endothermic effect that precluded accurate curve fitting. This was attributed to possible intramolecular interactions between the phosphonate groups of **2c**. These interactions could compete with the assembly of the molecular capsule. The formation of capsule **1•2d** shows the most favorable entropic contribution and the least favorable enthalpic term. Since the interaction between sulfonate and amidinium groups should be similar for calix[4]arene **2a** or thiacalix[4]arene **2d** with **1**, the more positive values observed for both ΔH° and ΔS° must be the result of a higher degree of solvation of the larger thiacalix[4]arene **2d**.

ITC measurements for assemblies **1•2a–c** were also performed in the presence of borate buffer ($Na_2B_4O_7$). The titrations were carried out at two different concentrations of borate buffer (c = 1.0 × 10^{-2} M and c = 0.33 × 10^{-2} M), leading to ionic strengths of I = 0.03 M and I = 0.01 M, respectively. Compared to **1•2a** and **1•2b**, assembly **1•2c** shows lower association constants (K_a = 4.5 × 10^4 M^{-1} or K_a = 6.7 × 10^3 M^{-1}, Table 2). This is most probably the result of a lower

Table 2 Association constants and thermodynamic parameters for the formation of assemblies **1a•2a–d** as determined by ITC in MeOH–H_2O (x_{water} = 0.4) at 298 K

Assembly	K_a/M^{-1}	ΔH°/kJ mol^{-1}	ΔS°/J K^{-1} mol^{-1}
1•2a[a]	(8.5 ± 1.4) × 10^6	14.1 ± 0.1	180 ± 2
1•2a[b]	(1.2 ± 0.1) × 10^6	−69.9 ± 0.2	−118 ± 1
1•2a[c]	(1.1 ± 0.1) × 10^5	−58.2 ± 0.4	−98 ± 2
1•2b[a]	(8.6 ± 1.9) × 10^6	17.1 ± 0.2	190 ± 2
1•2b[b]	(1.5 ± 0.1) × 10^6	−57.9 ± 0.1	−76 ± 1
1•2b[c]	(1.8 ± 0.1) × 10^5	−49.7 ± 0.4	−66 ± 2
1•2c[a]	—[d]	—[d]	—[d]
1•2c[b]	(4.5 ± 0.2) × 10^4	−55.3 ± 0.1	−96 ± 1
1•2c[c]	(6.7 ± 0.1) × 10^3	−40.9 ± 0.3	−63 ± 1
1•2d[a]	(1.0 ± 0.1) × 10^7	25.9 ± 0.1	221 ± 1

[a] Background electrolyte: 1.0 × 10^{-2} M Bu_4NClO_4 (I = 0.01 M). [b] Background electrolyte: 0.33 × 10^{-2} M $Na_2B_4O_7$ (I = 0.01 M, pH = 9.2). [c] Background electrolyte: 1.0 × 10^{-2} M $Na_2B_4O_7$ (I = 0.03 M, pH = 9.2). [d] Could not be determined because of the small heat effect.

degree of preorganization of **2c** due to the freedom of rotation around the C–P bond. Probably the formation of the capsule causes a reduction in the torsional entropy that overrides the gain in entropy, caused by the desolvation of the charged groups as reflected in the negative values for ΔS°.[31]

The enthalpograms for titrations performed in the presence of the two different background electrolytes (Bu_4NClO_4 *vs.* borate buffer) but at the same ionic strength ($I = 0.01$ M) show opposite signs of the heat effects, which indicates a change in the thermodynamics of association. Going from Bu_4NClO_4 to borate buffer the overall process occurring in solution changes from endothermic and entropy-driven to exothermic and enthalphy-driven with the overall curve showing a negative contribution both from ΔH° and ΔS° (Fig. 5). It is well-known that the heat effect varies depending on the particular electrolyte employed. Most probably the different solvation modes of the charged moieties are of importance for the differences in the thermodynamics of binding.

More interesting is the variation of the heat effect depending on the specific electrolyte concentration in solution. The association constant decreases on increasing the ionic strength. For example, for assembly **1•2a** K_a decreases from 1.2×10^6 M^{-1} at $I = 0.01$ M to $K_a = 1.1 \times 10^5$ M^{-1} at $I = 0.03$ M. Increasing the ionic strength in general weakens the electrostatic interactions. When a higher concentration of salt is present in solution, the Coulombic attractive forces between the two building blocks are considerably shielded and the binding is weakened. This is reflected in a less negative value of ΔH°. Although the observed K_a decreases upon increasing the salt concentration, a change in the mode of binding is not expected as no change in the stoichiometry of the interaction was observed.

Conclusions

The results presented demonstrate that multiple ionic interactions represent an excellent tool for the synthesis of molecular containers in polar solvents. Evidence for the formation of the 1 : 1 calix[4]arene based molecular capsules from a tetraamidinium calix[4]arene and different calix[4]arenes functionalized at the upper rim with sulfonate, carboxylic acid, or phosphonate moieties (**2a–c**) was provided by a combination of different techniques like ^{1}H NMR, ESI-MS, and ITC. The strength of the ionic interactions allows the molecular complexes to form in a highly polar solvent (MeOH–H_2O) and in the presence of a large excess of electrolyte (Bu_4NClO_4 and borate buffer). Differences in thermodynamics of binding were observed depending on the electrolyte present in solution. The assembly between the conformationally flexible thiacalix[4]arene **2d** and the tetraamidinium calix[4]arene **1** was also investigated. X-Ray structural analysis shows that the large thiacalix[4]arene **2d** in the solid state forms a three-dimensional network with **1**. In solution, the ^{1}H NMR upfield shifts and NOE connectivities observed for the protons of the alkyl side chain of **1** upon complex formation indicate that a well-defined **1•2d** molecular capsule is formed instead.

Acknowledgements

This work was financially supported by CW-NWO (project number JC 99538).

References

1 (*a*) D. J. Cram and J. M. Cram, *Container Molecules and Their Guests*, Royal Society of Chemistry, Cambridge, 1994; (*b*) D. A. Makeiff, D. J. Pope and J. C. Sherman, *J. Am. Chem. Soc.*, 2000, **122**, 1337–1342; (*c*) L. R. MacGillivray and J. L. Atwood, *Angew. Chem., Int. Ed.*, 1999, **38**, 1018–1033; (*d*) C. A. Schalley, A. Lützen and M. Albrecht, *Eur. J. Org. Chem.*, 2004, 1072–1080.
2 (*a*) J. Rebek, Jr., *Acc. Chem. Res.*, 1999, **32**, 278–286; (*b*) J. Rebek, Jr., *Chem. Commun.*, 2000, 637–643.
3 (*a*) For reviews on molecular capsules based on hydrogen bonds, see: M. M. Conn and J. Rebek, Jr., *Chem. Rev.*, 1997, **97**, 1647–1668; (*b*) F. Hof, S. L. Craig, C. Nuckolls and J. Rebek, Jr., *Angew. Chem., Int. Ed.*, 2002, **41**, 1488–1508.
4 (*a*) For reviews on molecular capsules based on metal ligand interactions, see: F. A. Cotton, C. Lin and C. A. Murillo, *Acc. Chem. Res.*, 2001, **34**, 759–771; (*b*) B. J. Holliday and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2001, **40**, 2022–2043; (*c*) S. Russel-Seidel and P. J. Stang, *Acc. Chem. Res.*, 2002, **35**, 972–983.
5 (*a*) R. Garcia-Zarracino and H. Hopfl, *Angew. Chem., Int. Ed.*, 2004, **43**, 1507–1511; (*b*) J. L. Atwood, L. J. Barbour and A. Jerga, *Chem. Commun.*, 2001, 2376–2377; (*c*) M. O. Vysotsky, I. Thondorf and V. Böhmer, *Chem. Commun.*, 2001, 1890–1891; (*d*) A. Shivanyuk and J. Rebek, Jr., *Chem. Commun.*, 2001, 2374–2375.
6 R. S. Castellano, S. L. Craig, C. Nuckolls and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2000, **122**, 7876–7882.
7 J. Kang, and J. Rebek, Jr., *Nature (London)*, 1997, **385**, 50–52.
8 (*a*) M. Yoshizawa, M. Tamura and M. Fujita, *J. Am. Chem. Soc.*, 2004, **126**, 6846–6847; (*b*) D. Rechavit, A. Scarso and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2004, **126**, 7738–7739; (*c*) R. G. Chapman and J. C. Sherman, *J. Am. Chem. Soc.*, 1995, **117**, 9081–9082; (*d*) A. Shivanyuk and J. Rebek, Jr., *Angew. Chem., Int. Ed.*, 2003, **42**, 684–686; (*e*) A. Shivanyuk and J. Rebek, Jr., *J. Am. Chem. Soc.*, 2002, **124**, 12074–12075.
9 A. Warshel, *Acc. Chem. Res.*, 1981, **14**, 284–290.
10 F. Diederich, in *Cyclophanes (Monographs in Supramolecular Chemistry)*, ed. J. F. Stoddart, Royal Society of Chemistry, Cambridge, 1991, ch. 2, pp. 52–105.
11 (*a*) S. B. Lee and J.-H. Hong, *Tetrahedron Lett.*, 1996, **37**, 8501–8504; (*b*) B. Hamilin, L. Jullien, C. Derouet, C. Hervé du Penhoat and P. Berthault, *J. Am. Chem. Soc.*, 1998, **120**, 8438–8447; (*c*) R. Fiammengo, P. Timmerman, F. de Jong and D. N. Reinhoudt, *Chem. Commun.*, 2000, 2313–2314; (*d*) T. Grawe, T. Schrader, M. Gurrath, A. Kraft and F. Osterod, *Org. Lett.*, 2000, **2**, 29–32; (*e*) H.-J. Kim, S. Sakamoto, K. Yamaguchi and J.-I. Hong, *Org. Lett.*, 2003, **5**, 1051–1054.
12 F. Corbellini, R. Fiammengo, P. Timmerman, M. Crego-Calama, K. Versluis, A. J. R. Heck, I. Luyten and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2002, **124**, 6569–6575.
13 (*a*) R. Zadmard, T. Schrader, T. Grawe and A. Kraft, *Org. Lett.*, 2002, **4**, 1687–1690; (*b*) R. Zadmard, M. Junkers, T. Schrader, T. Grawe and A. Kraft, *J. Org. Chem.*, 2003, **68**, 6511–6521.
14 (*a*) N. Iki and S. Miyano, *J. Inclusion Phenom. Macrocycl. Chem.*, 2001, **41**, 99–105; (*b*) N. Kon, N. Iki and S. Miyano, *Org. Biomol. Chem.*, 2003, **1**, 751–755.
15 R. Fiammengo, P. Timmerman, J. Huskens, K. Versluis, A. J. R. Heck and D. N. Reinhoudt, *Tetrahedron*, 2002, **58**, 757–764.
16 H. Matsumiya, Y. Terazono, N. Iki and S. Miyano, *J. Chem. Soc., Perkin. Trans. 2*, 2002, 1166–1172.
17 P. Molenveld, J. Engbersen and D. N. Reinhoudt, *Eur. J. Org. Chem.*, 1999, 3269–3276.
18 A. Arduini, A. Casnati, M. Fabbi, P. Minari, A. Pochini, A. R. Sicuri and R. Ungaro, *Supramol. Chem.*, 1993, **1**, 235–246.
19 H. Hioki, R. Nakaoka, A. Maruyama and M. Kodama, *J. Chem. Soc., Perkin Trans. 1*, 2001, 3265–3268.
20 E. Pinkhassik, V. Sidorov and I. Stibor, *J. Org. Chem.*, 1998, **63**, 9644–9651.
21 A. Ikeda and S. Shinkai, *J. Am. Chem. Soc.*, 1994, **116**, 3102–3110.
22 D. J. E. Spencer, J. B. Johnson, B. J. Johnson and W. B. Tolman, *Org. Lett.*, 2002, **4**, 1391–1393.
23 G. M. Sheldrick, *SHELXS86. Program for crystal structure determination*, University of Göttingen, Germany, 1986.
24 G. M. Sheldrick, *SHELXL-97-2, Program for refinement of crystal structures*, University of Göttingen, Germany, 1997.
25 A. L. Spek, *J. Appl. Crystallogr.*, 2003, **36**, 7–13.
26 P. D. Harvey, *Coord. Chem. Rev.*, 2002, **233**, 289–309.
27 (*a*) R. S. Garipati, *Tetrahedron Lett.*, 1990, **31**, 1969–1972; (*b*) L. Sebo and F. Diederich, *Helv. Chim. Acta*, 2000, **83**, 93–113.
28 R. J. Bernardino and B. J. Costa-Cabral, *J. Mol. Struct. (THEOCHEM)*, 2001, **549**, 253–260.
29 F. Corbellini, L. Di Costanzo, M. Crego-Calama, S. Geremia and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2003, **125**, 9946–9947.
30 (*a*) B. Linton and A. D. Hamilton, *Tetrahedron*, 1999, **55**, 6027–6038; (*b*) L. Sebo, B. Schweizer and F. Diederich, *Helv. Chim. Acta*, 2000, **83**, 80–92; (*c*) X. Salvatella, M. W. G. M. Peczuh, R. K. Jain, J. Sanchez-Quesada, J. de Mendoza, A. D. Hamilton and E. Giralt, *Chem. Commun.*, 2000, 1399–1400; (*d*) M. Berger and F. P. Schmidtchen, *Angew. Chem., Int. Ed.*, 1998, **37**, 2694–2696.
31 S. L. Hauser, E. W. Johanson, H. P. Green and P. J. Smith, *Org. Lett.*, 2000, **2**, 3575–3578.