Journal of Inclusion Phenomena and Macrocyclic Chemistry **46:** 161–166, 2003.

Solution and Solid-State Interactions between Para-sulphonato-Calix-[4]-Arene and Some Common Organic Biological Buffers

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(Received: 14 November 2002; in final form: 16 June 2003)

Abstract

The complexation of a series of organic buffers by *para*-sulphonato-calix-[4]-arene has been studied by ^{1}H NMR titration spectroscopy and X-Ray crystallography. The association constant range is observed from 9 mM for 2-[N-Morpholino]ethanesulfonic acid] (MES) to 6720 mM for (1,3-bis[tris(hydroxymethyl)-methylamino]-propane) (BTP). The structure of BTP with *para*-sulphonato-calix-[4]-arene shows the buffer molecule to be partially included in the host cavity. The packing arrangement shows the normal bilayer organic clay motif.

Introduction

The calix-[n]-arenes [1] are one of the three majors groups of organic macrocyclic host compounds in supramolecular chemistry [2]. Both their chemistry [3] and their complexation properties with respect to a wide range of ions [4] and small organic molecules [5] have been widely studied.

The first report on the biological activity of the calix-[n]-arenes was by Cornforth in 1955, concerning their anti-tubercular activity [6]. For more than 30 years following this the bioactivity of the calix-[n]-arenes remained largely unstudied, the recent resurgence of interest in this field started with work by Atwood on the chloride ion-channel blocking properties of the para-sulphonato-calix-[n]-arenes in 1996 [7]. In recent years work has been published on the use of calix-[n]-arenes as molecular skeletons for the development of bio-mimetic systems [8–10], for the study of their interactions with amino-acids [11], proteins [12–13] and DNA [14]. Of the calix-[n]-arenes the para-sulphonato-derivatives, with the high aqueous solubility, are the most widely studied in the context of intrinsic bio-activity. They have been shown to exhibit anti-thrombotic [15], anti-viral activity [16-17] and enzyme inhibition properties [18]. We [19-21] and others [22] have studied by a number of techniques their interactions with amino-acids and small peptides. We recently demonstrated, by electrospray mass-spectrometry, selectivity in their interaction with the ubiquitous proteins, the Serum Albumins [10]. One problem experienced by any group working in the field of the study of the interactions between the para-sulphonato-calix-[4]-arene and biological molecules lies in the need to work under physiological conditions of pH and ionic strength. Generally PBS has been used as the buffer of choice. [23]

We are interested in working with other biological buffers particularly in the case of experiments on cell culture in the presence of the para-sulphonato-calix-[4]-arene, where such buffers are more commonly used than PBS. In this paper we wish to report on studies using ^{1}H NMR of the interaction of the organic buffers; 3-[N,Morpholino]propanesulfonic acid (MOPS), Tris(hydroxymethyl)-aminomethane (Trometamol), [4-amino-4-(3-hydroxypropyl)-1,7-heptanediol] (Bis-homotris), (N-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) (TES), (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) (HEPES), 2-[N-Morpholino]-ethanesulfonic acid) (MES), Glycylglycine (GlyGly), Bis[2-hydroxyethyl]imino-Tris[hydroxymethyl]methane (Bistris), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), (1,3-bis[tris(hydroxymethyl)-methylamino]propane) (BTP), with para-sulphonato-calix-[4]-arene in solution. From the derived association constants we show that GlyGly and TES show effectively zero interactions and are thus suitable for use in buffering para-sulphonato-calix-[4]-arene for use in biological studies. The crystal structure of the complex between para-sulphonato-calix-[4]-arene (**1**) and Bis-Tris-Propane (BTP) shows that the observed interactions involve complexation of the buffer molecule in the calix-cavity. Here the BTP molecule is in fact too large to be fully included in the calix cavity and is symmetrically positioned with one half included and the second half directed outside the calix.

Experimental

Reagents

1 was synthesised according to the literature method [24]. The MOPS, CAPS, BTP, TES, HEPES, BisTris and MES

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were purchased from Sigma, Trometamol and Glycylglycine were purchased from Merck and BisHomoTris was purchased by Aldrich. The buffers were used without further purification.

Apparatus

^{1}H NMR spectra were collected at 25 ° on a Varian Unity 500 MHz Spectrometer.

^{1}H NMR analysis

The ^{1}H NMR titrations were carried out in 95% H_2O–5% D_2O [21]. The titration was carried out by introducing 300 μL of buffer solution (0.01 mol L^{-1}) in the range of pH 6 to 11 into NMR tubes and by adding increasing amounts of 0 to 700 μL of calixarenes solutions (0.04 mol L^{-1}), completing to 1 mL with de- ionised water purified with a Milipore MiliQ water system in order to obtain a resistivity of at least 18 MΩ cm. An NMR spectrum of each tube was recorded. A minimum of 15 points was obtained.

Determination of stability constants

Stability constants K_{ass} of calixarene complexes with buffer in solutions are determined by NMR spectrometry [25]. Association constants of the different complexes determined by non-linear square regression using Sigma Plot, rsqr > 0.999, limited displacement and maximum induced displacement of the protons of the guests.

X-ray crystallography

Intensity data for the complex **1**·2BTP·xH_2O were collected at 200 K on a Bruker SMART-APEX diffractometer using $Mo_{\kappa\alpha}$ radiation (λ = 0.7107 Å). Lorentz and polarization corrections were applied and the structure was solved by direct methods and Fourier techniques. Structure solution and refinement were based on $|F|^2$ All non-hydrogen atoms were refined with anisotropic displacement parameters. The H atoms of the C—H groups were fixed in calculated positions. One BTP molecule was found to be partially included in the calixarene and its NH and OH hydrogen atoms were located via difference Fourier map inspection and refined with riding coordinates and isotropic thermal parameters based upon the corresponding N and O atoms [U(H) = 1.2 U_{eq} (O, N)]. The second independent molecule of BTP in the asymmetric unit was observed to be disordered around a 2-fold position and it was refined with two equally occupied sets of coordinates. One water molecule was refined anisotropically with full occupancy while the other oxygen atoms were disordered over 17 general positions and refined with variable site occupation factors which added to a total of occupancy of 8.0025(1), and a common isotropic thermal parameter. All crystallographic calculations were conducted with the SHELXTL 6.10 program package.

Crystal data for **1**·2BTP·xH_2O, empirical formula $C_{50}H_{42}N_4O_{37}S_4$, M = 1419.12 g mol[1], triclinic, space group $P-1$, a = 13.5298(13), b = 14.3142(13), c = 16.9360(16) Å, $alpha$ = 104.192(2), β = 91.787(2), γ = 96.979(2) deg, U = 3150.0(5) Å^3, Z = 2, d_{calc} = 1.496 g cm^{-3}, μ = 0.255 mm^{-1}, $F(000)$ = 1460, $2\theta_{max}$ = 48.62° ($-16 \le h \le 13$, $-15 \le k \le 17$, $-20 \le l \le 16$). Final residuals (for 843 parameters) were $R1$ = 0.0895 and $wR2$ = 0.2339 for 4760 reflections with $I > 2\sigma(I)$ and $R1$ = 0.1647 and $wR2$ = 0.2694 for all 10929 data (R_{int} = 0.0635). Residual electron density was 0.718 and −0.542 e Å^{-3}.

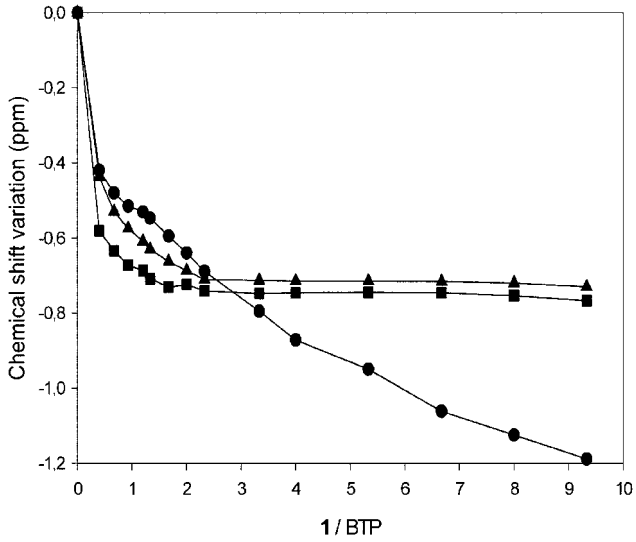


Figure 1. Proton chemical shift variation of BTP in presence of increasing concentration of **1** where —CH_2OH, —CH_2NH and —CH_2 of BTP, as shown in Table 1, are represented by (●), (■), (▲), respectively.

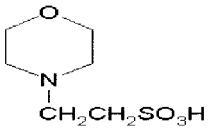
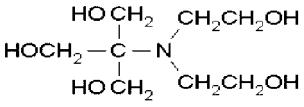
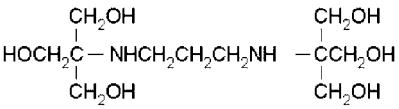
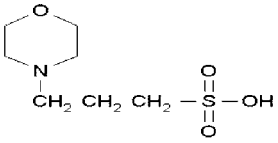
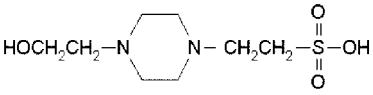
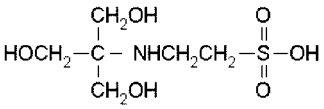
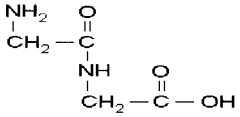
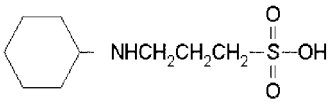
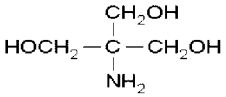
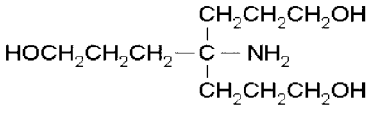
Results and discussion

In Table 1 are given the chemical structures and useful pH ranges for the 10 buffers studied in the current work, the buffers were chosen to cover the range of pH values from 5–11, and are considered to be representative of organic buffers used in biological studies. In Figure 1, is presented the ^{1}H NMR titration curve for the complexation of **1** with BTP. The signal of the protons undergoes the typical down field shift associated with complexation of organic molecules with **1** [22–23].

In Table 2 are given the values of the 1 : 1 association constants observed for the varians buffers with **1**. As with the complexation of amino-acids and peptides with **1**, there is no evidence for other stoichiometries.

Glygly and Trometamol show K_{ass} values of 13 and 10 M^{-1} with standard deviations higher than the observed association constants. We consider that for these buffers there is effectively no detectable interaction with **1**. Similar effective zero association constants were observed, using ^{1}H NMR titration experiments, by Ungaro [22] for the interactions of **1** with the amino-acids Alanine and Tyrosine. The useful pH are 7.5–8.9 for Glygly, 10.2–10.6 for Trometamol, thus within the typical physiological pH limits of 7.38–7.45, the buffer of choice for the measurement of interactions of **1**

Table 1. Chemical structures and useful pH ranges for the 10 buffers

Buffer	Nomenclature	Structure	pH range
MES	2-[N-Morpholino]-ethanesulfonic acid		5.5 to 6.7
BIS TRIS	(bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane)		5.8 to 7.2
BTP	(1,3-bis[tris(hydroxymethyl)-methylamino]propane)		6.3 to 9.5
MOPS	(3-[N-Morpholino] propanesulfonic acid		6.5 to 7.9
HEPES	(N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])		6.8 to 8.2
TES	(N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid		6.8 to 8.2
GLYGLY	glycylglycine		7.5 to 8.9
CAPS	3-(cyclohexylamino)-1-propanesulfonic acid		9.7 to 11.2
TROMETAMOL	Tris(hydroxymethyl)-aminomethane		10.2 to 10.6
BISHOMOTRIS	[4-amino-4-(3-hydroxypropyl)-1,7-heptandiol]		

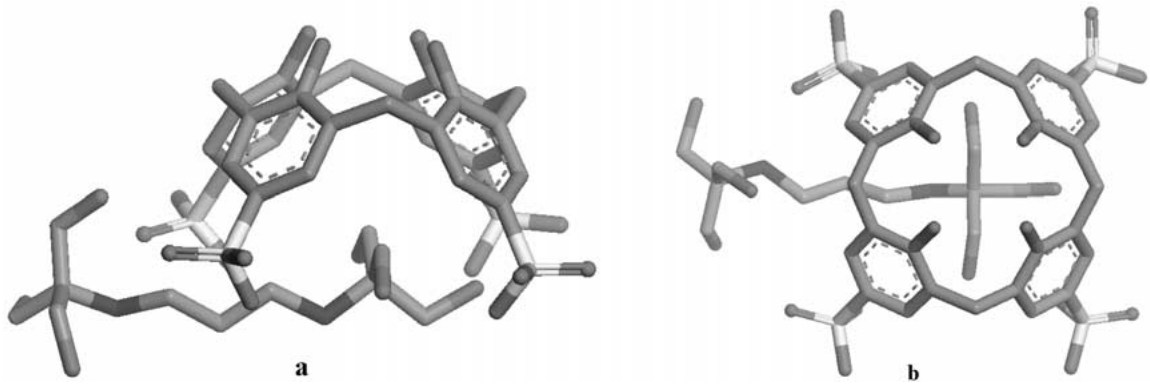


Figure 2. Crystal structure of the complex between para-sulphonato-calix-[4]-arene (**1**) and bis-tris-propane (BTP) down the *x*-axis (a) and down the *y*-axis (b).

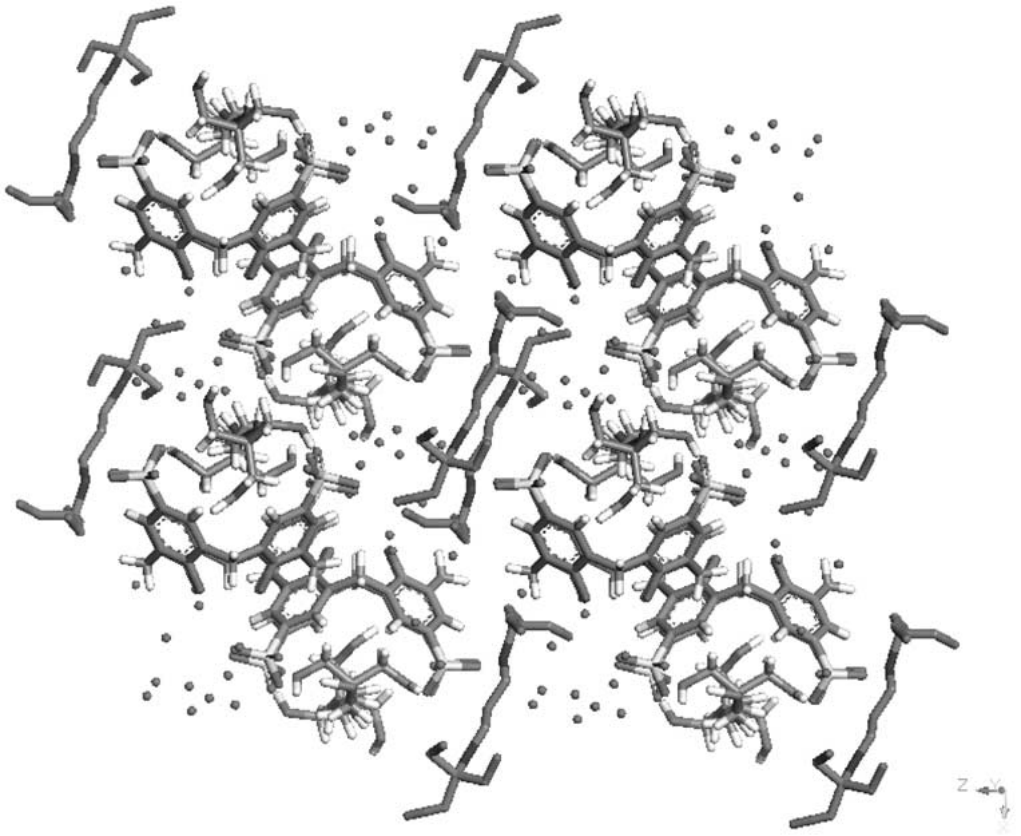


Figure 3. View of the packing of **1**·2BTP·9H_2O down the *y*-axis illustrating the hydrophilic layer formed by the included dimmers of BTP and how the non-included moieties disrupt the bilayer motif (hydrogen atoms of water molecules and non-included BTP molecules are omitted for clarity).

with biological molecules is Glygly, where interference from the buffer will be minimised.

TES shows low but significant 1 : 1 K_{ass} values with **1** in the range of 30 M^{-1}. While the values are low in absolute terms, given the likely differences in concentration of **1** and bioactive molecules compared to the typical buffering concentration of 0.01 M, significant interference due to the buffer can be expected in experimental determination of molecular interaction.

For a third group of buffers, comprising BisTris, MOPS, MES and BisHomoTris, the 1 : 1 K_{ass} with **1** vary between 102 and 527 M^{-1}. The values show reasonably strong interactions in these cases, the buffers here have useful pH ranges of 6–8 representing, unfortunately, the most usual physiological pH conditions.

In the case of HEPES and CAPS, the associations constants values K_{ass} are higher than those observed with others buffers. The association constants values are 1598 M^{-1} and 2056 M^{-1} for HEPES and 978 M^{-1} and 1620 M^{-1} for CAPS. There are often large divergences in the K_{ass} values obtained for different protons within a given buffer molecule; this reflects a partial inclusion with one part of the molecule within the cavity and the other part situated outside in a hydrated state. This is particularly evident for MES, where the K_{ass} values are 9 and 527 M^{-1} and for BTP 322 and 6720 M^{-1}.

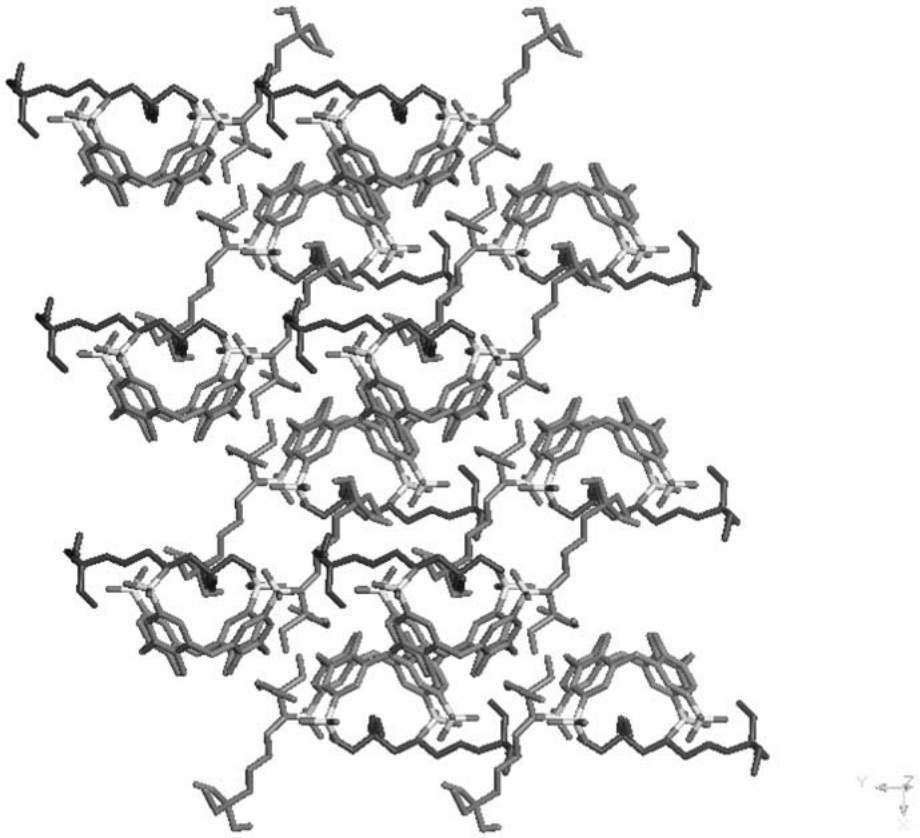


Figure 4. View of the packing of **1**·2BTP down the *z*-axis illustrating how the non-included dimmers of BTP non-included (distinct from the included buffer molecules shown in dark grey) traverse the bilayers (hydrogen atoms and water molecules are omitted for clarity).

Table 2. Association constants values K_{ass} (M^{-1}) of the different complexes determined by non-linear square regression using *Sigma Plot*, rsqr > 0.999 and maximum induced displacement of the protons of buffers

Buffer	K_{ass} (M^{-1})	RSD (%)
MES	9 ± 2	22
	527 ± 75	14
BISTRIS	134 ± 24	18
BTP	322 ± 27	8
	6720 ± 4731	70
MOPS	102 ± 19	19
	451 ± 79	18
HEPES	1598 ± 233	15
	2056 ± 378	18
TES	20 ± 4	20
	34 ± 11	32
	50 ± 22	44
GLYGLY	13 ± 65	–
CAPS	978 ± 168	17
	1620 ± 190	12
TROMETAMOL	10 ± 58	–
BISHOMOTRIS	125 ± 54	43
	345 ± 31	9
	519 ± 49	9

Table 3. Selected hydrogen bond distances (in Å) between the oxygen atoms of the two BTP molecules and sulfonate oxygen, hydroxy and nitrogen atoms of the neighbouring calixarenes, BTP and water molecules

BTP incl	Å	BTP ext	Å
$O_{100}\cdots O_{12}S_1$ (incl)	2.958	$O_{18a}\cdots O_{43}S_4$	2.961
$O_{101}\cdots O_{12}S_1$ (incl)	3.161	$O_{18b}\cdots O_{43}S_4$	2.345
$O_{102}\cdots O_{23}S_2$ (incl)	2.767	$O_{19b}\cdots O_{22}S_2$	3.065
$O_{109}\cdots O_{11}S_1$ (adj)	2.659	$O_{21a}\cdots O_{13}S_1$	2.942
$O_{109}\cdots N_{100}$ (BTP'incl)	2.821	$O_{21b}\cdots O_{13}S_1$	2.470
$O_{108}\cdots O_{11w}$ (water)	2.495	$O_{20a}\cdots O_{19a}$ (BTP'ext)	2.585
$O_{110}\cdots O_{2w}$ (water)	2.750	$O_{20b}\cdots O_2$(H)C [4]	3.164

Thus, the choice of PBS as a standard buffer, initially dictated by the absence of interfering proton signals in 1H NMR experiments [22] dearly justified in terms of removing competing buffer-**1** interactions in the measurement of complexation between **1** and biological molecules.

The largest stability constant observed between **1** and BTP (K_{ass} = 6720 M^{-1}) is reflected in the obtention of a crystalline complex **1** : 2 BTP in H_2O by slow evaporation of a solution of the complex.

The molecular structure is given as two orthogonal views in Figures 2a and 2b. Two independent molecules of BTP are involved in the complex: one included and one exterior to the molecular cavity; similar solid-state behaviour was observed in the complex of **1** with lysine [26]. In this case,

contrary to the amino acid, the included BTP is too large for the whole molecule to fit into the molecular cavity of **1** and results in a disrupted bilayer formed now from back to back dimeric units of included molecules of BTP. The included moieties are hydrogen bonded to two sulphonate groups of the complexed calixarene while the external part interacts with a neighbouring calixarene of the same layer and with a parallel BTP molecule included by an adjacent layer of calixarenes (see Table 3 for selected distances). These dimers connecting the sulphonate oxygen atoms of two up and two down calixarenes through two included molecules of BTP form the hydrophilic layer. Because the BTP molecules are only partially included in the cavities of each calixarene, the hydrophobic layer is disrupted by the presence of a non-included BTP moiety between the sulphonate groups of two adjacent calixarenes along the axis of the BTP molecule (y-axis) in a similar situation as for the lysine traversing the hydrophobic layer and spanning the bilayer, with the difference that the included BTP do not traverse the hydrophobic layer, and separate only the sulphonate groups of the same side of the hydrophilic layers (Figure 3). The second, non-included, molecule of BTP is oblique with respect with the plane of the bilayer (angles of 33.2° and 5.65° with respect with the axis of the calixarenes in the planes xy and xz respectively). Each non-included BTP interacts by hydrogen bonding with the sulphonate oxygen atoms and with the hydroxy oxygen of two successive layers of calixarenes. The water molecules are situated in the hydrophilic space left by the buffer molecules, they interact with the sulphonate and hydroxyl groups of the calixarenes and the OH of both buffer molecules and with themselves with distances O···O in a range of 2.50 to 3.08 Å (see Table 3 for selected distances).

Moreover, each non-included molecule of BTP interacts with another BTP from the next bilayer by H-bond between two hydroxy groups ($d_{O\cdots O} = 2.58$ Å), forming a dimer, which traverses the bilayer along an oblique direction. The dimeric connection between the two BTP crosses the hydrophobic layer, while the rest of each molecule threads through the hydrophilic part (Figure 4). The overall situation is thus very similar to that of complex of **1** with lysine, but in this case, the non-included dimeric units and the included dimers are pseudo-orthogonal with each other and span the bilayers in two directions. Finally the widths of the hydrophilic and hydrophobic layers are 7.67 and 5.74 Å, respectively.

Thus the wide divergence in the K_{ass} values observed between **1** and BTP are explained in terms of the geometry of the complex.

Conclusion

We have shown that many common organic biological buffers can interact with para-sulphonato-calix-[4]-arene both in solution and solid phase. For the typical physiological pH range around pH 7, the buffer of choice will be GlyGly, which shows effectively a zero K_{ass}. The crystal structure of the **1**·2BTP·9H_2O complex shows partial inclusion of BTP and some disruption of the typical "organic clay" structure of para-sulphonato-calix-[4]-arene.

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