Designer amphiphiles based on *para*-acyl-calix[8]arenes

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The synthesis of a series of fully *O*-derivatised *para*-acyl-calix[8]arenes is described, where the acyl function is either octanoyl or hexadecanoyl. The groups attached at the phenolic face are, carboxymethoxy (anionic), carboxypropoxy (anionic), 4-sulfonatobutoxy (anionic), ethoxycarboxymethoxy (neutral), ethoxycarboxypropoxy (neutral), 2-methoxyethoxy (neutral) and 2-(2-methoxy)diethoxy (neutral). The use of specific synthetic routes has allowed complete substitution in high yields for all the compounds obtained. The interfacial properties of the compounds have been studied and stable monolayers have been obtained for certain compounds in the series having *para*-octanoyl substituents; all compounds studied in the series having *para*-hexadecanoyl substituents formed stable monolayers at the air–water interface. The interactions between *O*-4-sulfonatobutoxy-*para*-ocatanoylcalix[8]arene and a series of serum albumins have been studied by dynamic light scattering and specific adsorption of the calix-[8]-arene derivative onto the proteins observed. The anionic derivatives *O*-4-sulfonatobutoxy-*para*-ocatanoylcalix[8]arene and *O*-carboxymethoxy-*para*-ocatanoylcalix[8]arene have been shown to possess anticoagulant properties but to have no haemolytic toxicity.

Introduction

The calix[*n*]arenes are amongst the most widely studied organic macrocyclic host compounds.[1] They are synthesised size selectively in high yields from cheap and readily available starting materials, and even in the laboratory kilogram quantities can be prepared in a day. Given this ease of preparation and the fact that the chemistries at the *para*-position and the phenolic face are radically different, and thus do not require use of protecting groups, it is not surprising that a truly wide range of calix[*n*]arene derivatives is available for study.[2]

The complexation properties of the calix[*n*]arenes with regard to a wide range of substrates, including cations,[3] anions,[4] small organic molecules,[5] nucleotides,[6] DNA,[7] amino acids,[8] peptides[9] and proteins,[10,11] have been studied. Such complexation has been studied in the solid-state,[12] in solution,[13] as solid-substrate interactions,[14] in the gas-phase,[15] at the air–liquid interface[16] and in colloidal suspension,[8] by a wide range of methodologies.

The *para*-acyl-calix[4]arenes were first described by No and Kim in 1998 *via* a Fries rearrangement of the corresponding esters,[17] and were prepared directly *via* the Friedel–Crafts acylation by Shinkai *et al.* in 1991.[18]

We have carried out extensive studies on these molecules.[19] The *para*-acyl-calix[4]arenes have been shown to form stable monolayers at the air–water interface,[20] where they can interact with various cations and anions.[21]

Of particular interest is the capacity of these molecules to form solid lipid nanoparticles, SLNs, using the solvent diffusion method.[22] The properties of these SLNs have been widely studied, including their very high stability, the capacity to interact with serum albumins with formation of a monolayer of protein around the SLNs,[8] that they may be incorporated without aggregation into gels.[23] It has been shown however that their stability with regard to freeze drying requires use of cryo-protectants for re-suspension of the SLNs.[24]

The solid-state properties of both the *para*-acyl-calix[4]arenes and their derived SLNs are quite remarkable.[25] Single crystal to single crystal guest exchange has been observed for non-porous crystalline states, and indeed this exchange can occur even with large guest molecules, for example stilbenes.[26] Encapsulation within the van der Waals nano-capsules formed by these molecules leads to protection against UV photodegradation.[27] Solvent molecules can be swept out of both the nanocapsules and the SLNs by use of Xenon gas, with uptake of Xenon observed.[28]

Having been interested in the biological properties of the calix[*n*]arenes over a number of years, we have observed that in general the biological activity, as measured by complexation of amino acids[29] or peptides,[9] or for direct activity such as anticoagulant behaviour,[30] increases with increase in the size of the macrocycle. Thus, investigation of the synthesis and properties of the *para*-acyl-calix[6]arenes and *para*-acyl-calix[8]arenes has been for some time a major project within our group.

Interestingly, complete acylation at the aromatic face of *para*-acyl-calix[6]arene has proved almost impossible to achieve, in our hands, with highly complex mixtures having esterification at the phenolic face and incomplete *para*-substitution being obtained. Although, Shinkai *et al.* first reported the synthesis of *para*-acyl-calix[8]arenes in 1993,[31] it was only recently that we achieved clean total *para*-substitution for calix[8]arene, using acyl chains of eight

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or more carbon atoms,[32] and thus opening up a route to novel amphiphilic systems derived from substitution at the phenolic face of the *para*-acyl-calix[8]arenes.

In this paper we will describe the synthesis of two novel series of *para*-acyl-calix[8]arene derivatives having neutral or anionic functional groups attached at the lower rim. They are described as designer molecules in that we demonstrate that a wide range of derivatives can be produced by selective coupling of desired functions on a basic skeleton. These molecules show, in certain cases, formation of stable monolayers at the air–water interface. Initial studies on their biological activity, for example their capacity to complex proteins such as serum albumins, have been demonstrated by use of dynamic light scattering. Further observations have shown that certain anionic derivatives show anticoagulant behaviour. Finally the initial studies of haemolytic activity show that in general these molecules are non-toxic.

Results and discussion

Synthesis

The molecular structures of *para*-acyl-calix[8]arene derivatives prepared during the course of this study are given along with their numeration, in Scheme 1. Molecules **3a** and **3b**, **4a** and **4b**, **5a** and **5b**, **6a** and **6b** and **7a** and **7b** carry neutral functions at the phenolic face; molecules **8a**, **9a** and **9b** and **10a** and **10b** carry anionic functions.

Attempts to obtain cationic ammonium derivatives *via* alkoxynitrile derivatives, Scheme 1, yielded the desired intermediate butyronitrile derivative **3a** and **3b**, in good, 70%, yields with full substitution at the phenolic face by reaction of bromobutyronitrile with **2a** or **2b** in acetone with potassium carbonate as base. However all attempts at reduction of these derivatives yielded intransigent incomplete reaction mixtures, which have proved to be totally inseparable. Interestingly use of either bromoacetonitrile or bromopropionitrile as the alkylating agent leads to decomposition and full substitution at the phenolic face is not observed.

The synthetic route to molecules **4a** and **4b**, and **5a** and **5b** is *via* treatment of the parent *para*-acyl-calix[8]arene in acetone with 1.5 equivalents per hydroxyl function of 2-bromoethyl methyl ether or 1-bromo-2-(2-methoxyethoxy)ethane using 1.5 equivalents per hydroxyl function of potassium carbonate as base, with potassium iodide as a catalytic halogen transfer agent under reflux during 48 hours. After this time the same quantities of alkylating agent, base and potassium iodide were added to the reaction mixture which was then treated under reflux during 72 hours. For all four derivatives the reaction was complete after this time and the pure product could be isolated in good yields, **4a** 62%, **4b** 72%, **5a** 65% and **5b** 70%. A very recent paper, by Hii *et al.*,[33] treats the synthesis of macrocyclon analogues, *i.e. tert*-butyl- or *tert*-octylcalix[8]arene derivatives with ethylene glycol chains of at least three ethylene glycol units at the phenolic face. The authors found that, in contrast to this work where clean substitution is obtained with potassium carbonate as the base, for the longer ethylene glycol groups the use of caesium carbonate as a base is required for complete substitution.

The fact that we have obtained clean substitution with K_2CO_3 conflicts with the postulate that a template effect arises with caesium as a base or may best be explained by the acyl function at the *para*-position playing a strong role in determining the substitution mechanism at the phenolic face.

The *O*-alkoxy ester derivatives **6a** and **6b**, and **7a** and **7b**, were prepared by the treatment of **2a** or **2b** with 1.5 equivalents per hydroxyl group of ethyl bromoacetate or ethyl bromobutyrate, using 1.5 equivalents per hydroxyl function of potassium carbonate as base and potassium iodide as a halogen transfer agent in acetone under reflux during 48 hours; after this time total substitution of the phenolic hydroxyl groups had occurred and the products were obtained in good yields, 70–80%. However when the same synthesis was attempted using ethyl-4-bromoproprionate, incomplete substitution occurred even after addition of a second round of alkylating agent and base, and use of higher ratios of alkylating agent led to decomposition. In our hands it has so far proved impossible to separate the mixtures obtained from the reactions and thus the *O*-propoxy derivatives have as yet proved elusive.

Treatment of **2a** with excess of 1,4-butane sultone in tetrahydrofuran with excess sodium hydride as base under reflux, and with five repeated additions of base and 1,4-butane sultone over a 48 hour period, allowed isolation of the anionic *O*-4-sulfonatobutoxy derivative **8a**. The use of 1,3-propane sultone, shown by Shinkai *et al.* to alkylate the calix[*n*]arenes,[34] in our hands yielded only partial substitution of **2a** and **2b** and no fully substituted derivatives could be obtained. As noted by Hii *et al.*,[33] ES-MS or MALDI TOF is the methodology of choice both for clear identification of the products and more particularly for ascertaining the degree of substitution obtained. Given the high molecular weights of the products we have used exclusively MALDI TOF to determine product purity; all products show only the parent peaks with no undersubstitution or degradation of the products, as shown in Fig. 1.

In contrast to Hii *et al.* we observed no adduct formation with the MALDI matrix. ^{1}H NMR showed the bridging methylene protons between 4.14–4.04 ppm as a singlet, which implies that the molecules are not in the cone conformation that was observed for the non-substituted derivatives **2a** and **2b**, hence removal of possible hydrogen bonding groups at the phenolic face leads to conformational mobility. The ^{13}C NMR showed the bridging methylene carbon atoms between 32.4–31.5 ppm.

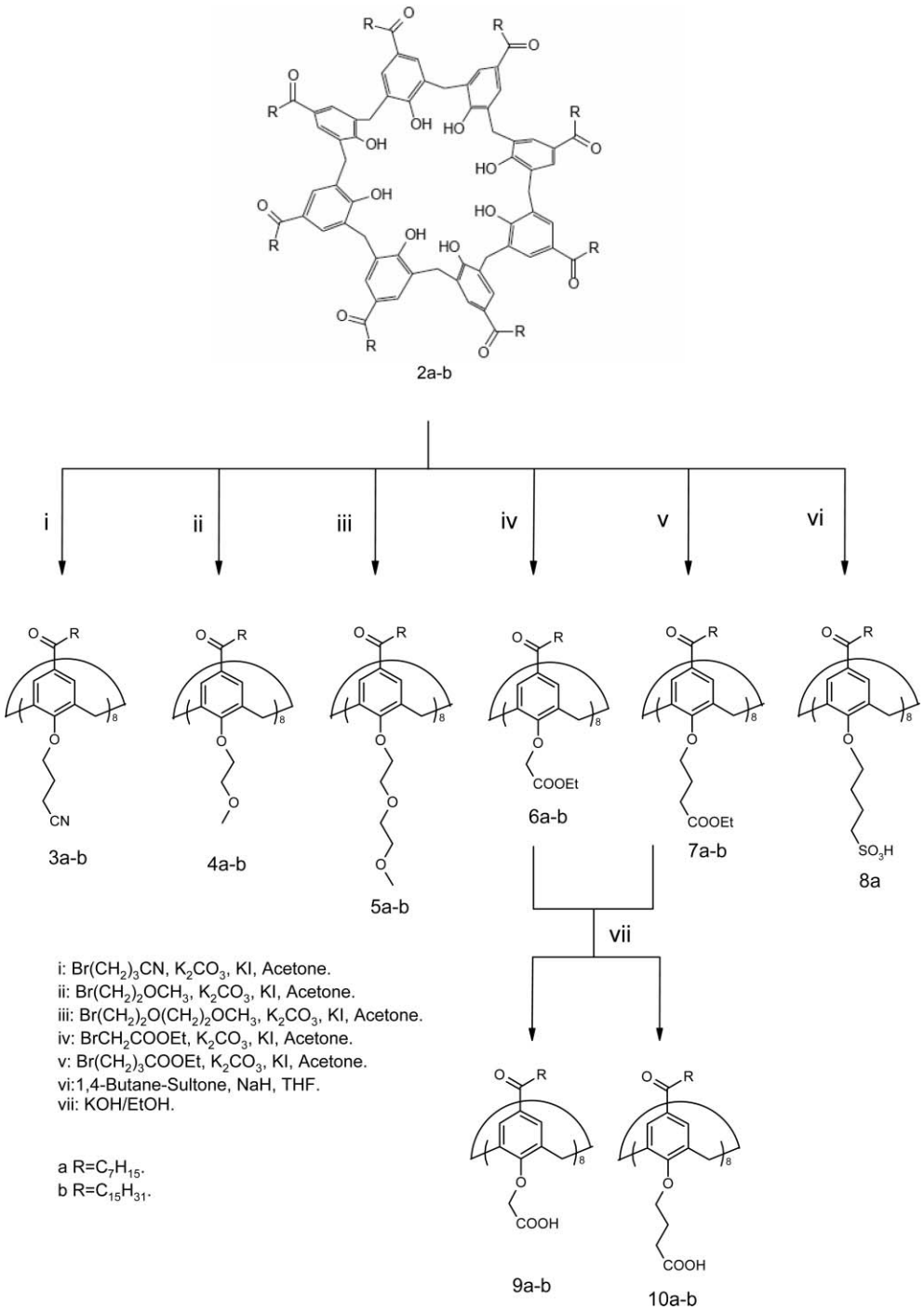
Interfacial assembly

For both series of derivatives the formation of monomolecular monolayers at the air–water interface has been studied by Langmuir compression isotherms and Brewster angle microscopy.

The isotherms are given in Fig. 2 and 4 respectively for the *para*-octanoylcalix[8]arene and *para*-hexadecanoylcalix[8]arene derivatives and the isotherm data is summarised respectively in Table 1 and 2. Brewster angle microscopy images are given in Fig. 3 and 5 respectively for certain *para*-octanoylcalix[8]arene and *para*-hexadecanoylcalix[8]arene derivatives.

No isotherm data were obtained for **8a** which shows aqueous solubility in spite of the presence of eight octanoyl chains in the *para*-position. The carboxylic acid derivatives **9a** and **10b** also present very low aqueous solubilities and hence care must be taken in considering the isotherm data.

For the *para*-octanoyl calix[8]arene derivatives there are considerable problems of reproducibility with variations in the values



Scheme 1 Synthetic routes to *para*-acyl-calix[8]arene derivatives.

observed of up to 30%; the values of area and collapse pressures given and the isotherms shown correspond to the highest values and were repeatable over about 50% of the compression isotherms.

In a detailed study of how conformational changes in the simplest amphiphilic calix[8]arene, *para-tert*-butylcalix[8]arene,[35] Martin-Romero *et al.* showed that compression derived changes from intra- and intermolecular hydrogen bonding in *para-tert*-butylcalix[8]arene to hydrogen bonding interactions with water were reasons for isotherm modification. However it was also noted that the nature of the spreading solvent,[36–38] spreading speed and compression speed played roles in the non-reproducible nature of the behaviour of amphiphilic calix[8]arenes at the air–water interface.[30] The situation with the *para*-octanoyl calix[8]arene derivatives is further complicated by their self-organisational behaviour.[34]

As can be seen from Fig. 2 all the molecules in the *para*-octanoyl-calix[8]arene series, form films at the air-water interface. A number of points are to be noted; the parent compound **2a** shows the highest apparent molecular area with an A_{coll} value of 236 Å^2, and a collapse pressure of 36.4 mN m^{-1}. For the other molecules of this

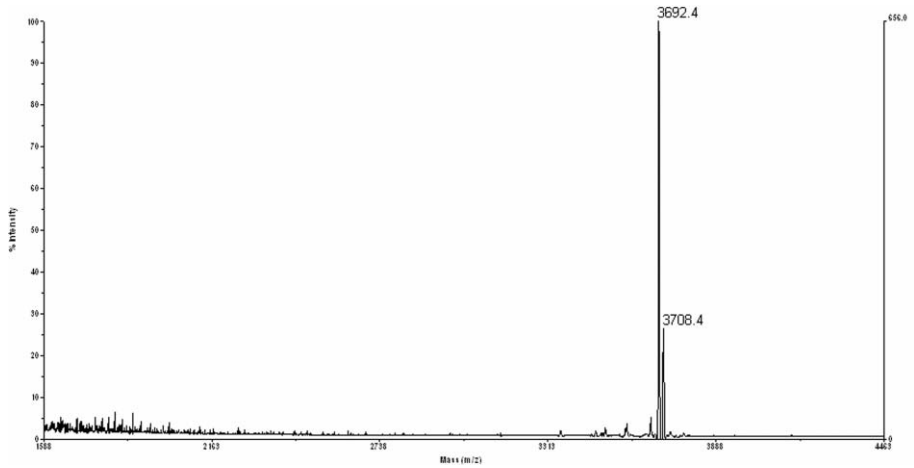


Fig. 1 Mass spectra of compound **7b**; 3692.4 = **7b** + Na^+, 3708.4 = **7b** + K^+.

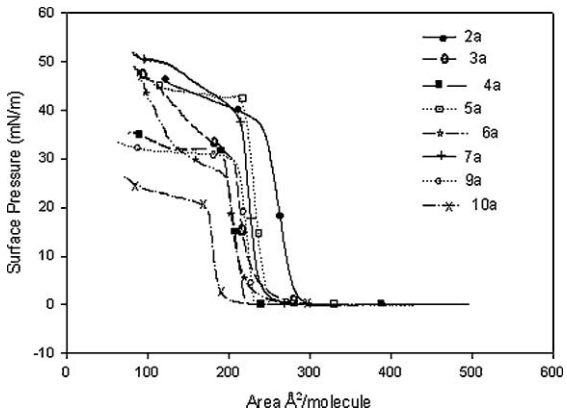


Fig. 2 Compression isotherms for the *para*-octanoylcalix[8]arene derivatives.

Table 1 Isotherm data for the *para*-octanoylcalix[8]arene derivatives

	Π_{coll}/mN m^{-1}	A_{coll}/Å^2	A_{lim}/Å^2	A_0/Å^2	A_1/Å^2	Cs^{-1}
2a	36.4	236	281	330	290	254
3a	30.3	203	226	315	273	316
4a	33.2	185	219	250	220	224
5a	42.9	210	245	260	249	359
6a	27.3	191	218	298	251	236
7a	39.8	206	237	295	258	368
9a	30.1	201	231	260	231	314
10a	20.5	168	188	270	201	257

A is the area in Å^2 per molecule, Π is the surface pressure in mN m^{-1}, and Cs^{-1} is the compressibility modulus at 20 °C. Π_{coll} is the pressure collapse, A_{coll} is the area collapse, A_{lim} is the extrapolated molecular area, A_0 is the apparent molecular area at $\pi = 0$, A_1 is the apparent molecular area at $\Pi = 1$ mN m^{-1}.

series the collapse areas are generally in the range 180 Å^2–210 Å^2, while **5a** and **7a**, having respectively *O*-butoxyethyl ester and *O*-diethoxymethyl ether functions, show respectively high collapse pressures of 42.9 and 39.8 mN m^{-1}. The other derivatives have collapse pressures clustered around 30 mN m^{-1}. Compound **9a**, which has an *O*-propoxycarboxylic acid function, shows very low collapse area and pressure, respectively 168 Å^2 and 20.5 mN m^{-1}.

Table 2 Isotherm data for the *para*-hexadecanoylcalix[8]arene derivatives

	Π_{coll}/mN m^{-1}	A_{coll}/Å^2	A_{lim}/Å^2	A_0/Å^2	A_1/Å^2	Cs^{-1}
2b	38.0	134	152	196	184	211
3b	36.1	171	217	253	233	180
4b	36.4	163	212	251	225	165
5b	47.9	174	239	384	296	176
6b	33.4	221	256	330	285	252
7b	35.3	154	200	310	277	131
9b	30.8	124	154	176	161	183
10b	35.9	184	228	312	238	157

Area in Å^2 per molecule, Π collapse and Cs^{-1} in mN m^{-1} at 20 °C. Π_{coll} is the pressure collapse, A_{coll} is the area collapse, A_{lim} is the extrapolated molecular area, A_0 is the apparent molecular area at $\pi = 0$, A_1 is the apparent molecular area at $\pi = 1$, Cs^{-1} is the compressibility modulus.

All compounds have compressibility indices, Cs^{-1}, in the range typical of liquid phases, ranging from 224 to 368.

The fact that quite large functional groups have been added to the phenolic face and yet smaller apparent molecular areas are observed suggests that there may be formation of three-dimensional films at the aqueous subphase.

Fig. 3 shows Brewster angle microscopy images of the interfacial assemblies formed by **3a**, **4a** and **5a**. The images were obtained after spreading of solutions of the derivatives, and are thus at 0 mN m^{-1} pressure and apparent molecular areas greater than 500 Å^2. For each system the left hand images show the pure water interface before spreading, the images were all taken at the same place on the surface and at time intervals of 1 minute. As can particularly be seen in Fig. 3 (**5a**), the films are rigid and immobile at the surface, with very high contrast levels between features, suggesting that an almost solid three-dimensional film may well be present. The films contain circular holes of approximately 600 μ in diameter. Such self-assembly is undoubtedly a major factor in the variability of the Langmuir isotherms and also in the apparently smaller molecular areas observed for the derivatives of *para*-octanoylcalix[8]arene.

For the compounds bearing hexanoyl chains at the *para*-position, the parent compound *para*-hexadecanoylcalix[8]arene with the observed apparent molecular area of 134 Å^2 is the smallest collapse area in the series of derivatives (Fig. 4). Thus in the series with longer acyl chains substitution at the phenolic face does

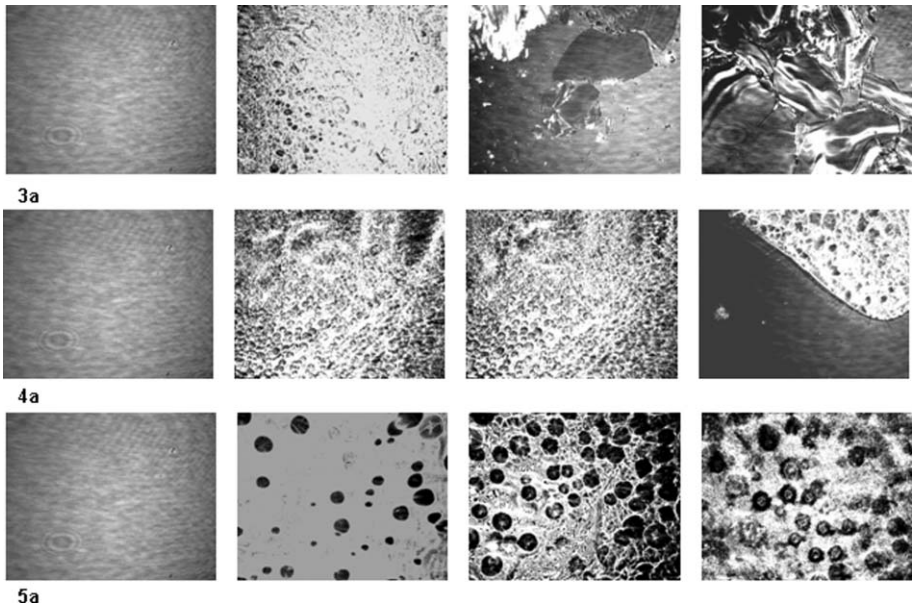


Fig. 3 Brewster angle images of films of **3a**, **4a** and **5a** spread at the air–water interface. The top left hand image is that of pure water and the images were taken sequentially at 1 minute intervals after deposition. The images are taken at 0 mN m^{-1} pressure and apparent molecular areas >500 $Å^2$, image sizes are 6.4 mm × 4.8 mm and resolution is 20 μ.

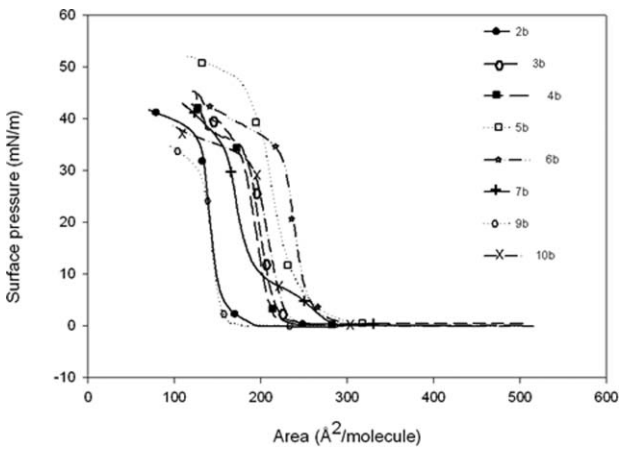


Fig. 4 Compression isotherms for the *para*-hexadecanoylcalix[8]arene derivatives.

lead to increased molecular areas. The ethoxycarbonylmethoxy derivative, **6b**, shows the largest molecular area of 221 $Å^2$, while for the propoxy analogue **7b** a much smaller molecular area of 154 $Å^2$ is observed. That the derivative with the shorter alkyl chain in the substituent group has a greater apparent molecular area may be due to the hydorophobic alkyl chains folding back into the cavity in the case of **7b**. Compound **7b** shows an apparent phase change from expanded liquid to condensed liquid phases occurring at a molecular area of 260 $Å^2$ and a surface pressure of 6 mN m^{-1}.

For the two derivatives bearing ethylene glycol derivatives at the phenolic face, **4b** has a smaller apparent molecular area, 163 $Å^2$, than **5b**, 174 $Å^2$. As might be expected the presence of a second ethylene glycol unit in the case of **5b** leads to a substantial increase in the collapse pressure observed (47.9 mN m^{-1}).

The *para*-hexadecanoylcalix[8]arene derivatives have compressibility indices, Cs^{-1}, in the range typical of liquid phases, ranging from 131 to 252, and which are generally lower than those observed for the corresponding *para*-octanoylcalix[8]arene derivatives.

Brewster angle microscopy of the films in this series (Fig. 5) shows, initially, liquid phase films, for which evaporation of the spreading solvent and organisation of the film is accompanied by the disappearance of fluid areas. Over a period of 5 minutes a slow crystallisation in two dimensions is observed, to yield fibrous structures of 35 μ in thickness and 400 μ in length, as can be seen in the final images in the two sets of images.

In general, it would appear that for the *para*-hexadecanoylcalix[8]arene derivatives the behaviour at the air–water interface is closer to that of typical amphiphilic calix[*n*]arenes than for the *para*-octanoylcalix[8]arene derivatives, but even for these derivatives there is considerable deviation from the formation of true monomolecular films. The conformational flexibility of the *para*-acyl-calix[8]arene skeleton coupled with the ability of the substituent groups to, probably, fold into the macrocycle makes it unlikely that any of the molecules studied will orient with the acyl chains and aromatic groups perpendicular to the air–water interface.

Biological properties

A series of preliminary studies have been undertaken with certain of the molecules of series a, the *para*-octanoylcalix[8]arene derivatives, and particularly with regard to **8a** which presents reasonable aqueous solubility and for which the presence of the sulfonate functions suggests analogy with the *para*-sulfonatocalix[*n*]arenes and by extension with the glycosylaminoglycans. A recent article by Mecca *et al.* has inverted this strategy to create cationic calix[8]arene derivatives for heparain recognition.[39]

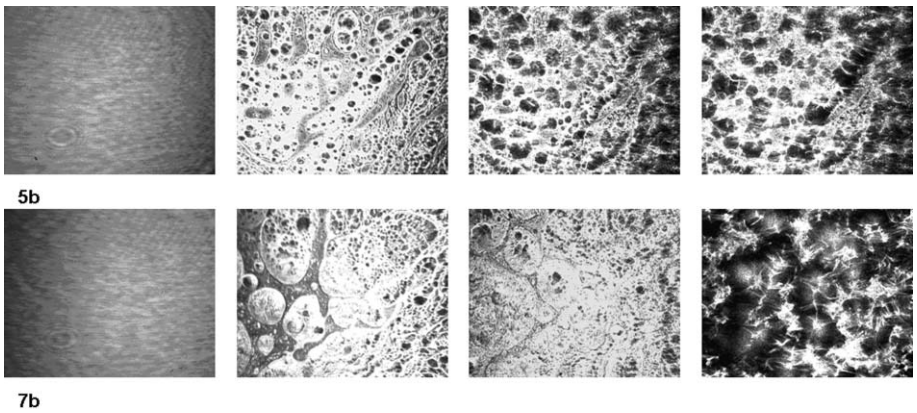


Fig. 5 Brewster angle images of films of **5b** and **7b** spread at the air–water interface. The top left hand image is that of pure water and the images were taken sequentially at 1 minute intervals after deposition. The images are taken at 0 mN m^{-1} pressure and apparent molecular areas >500 $Å^2$, image sizes are 6.4 mm × 4.8 mm and resolution is 20 μ.

Serum albumin interaction

We had previously investigated the interaction between various *para*-sulfonatocalix[*n*]arene derivatives and bovine serum albumin using electrospray mass spectrometry and it proved possible even to determine association constants using this methodology.[40]

However, under the same experimental conditions as used for the study of the interaction of the *para*-sulfonatocalix[n]arenes with bovine serum albumin, in the case of **8a** no peaks corresponding to the formation of a complex were observed in the mass spectrum. In place of this a clear decrease in the intensity of the peak at 66 kDa corresponding to BSA was observed, while the intensity of a secondary protein peak at 67 kDa remained unchanged, thus there is a clear interaction between **8a** and BSA but either the resultant m/z is out of the range of the electrospray mass spectrometer, which has a maximum m/z of 2000 or there is removal of BSA from the ionisation process. As no evident precipitation was observed, dynamic light scattering was used to study the change in the size of a series of serum albumin proteins in the presence of **8a**. The results are given in Table 3.

Interestingly **8a** itself is apparently present as a colloidal suspension with a monodisperse diameter of 235 nm. Lysozyme was used as a probe for non-specific interactions, and here, while the observed diameter increased considerably from 2.6 nm to 46 nm, no larger objects were observed. This change suggests the oligomerisation of lysozyme occurs in the presence of **8a** but that no floculation occurs. For all of the serum albumins, in the presence of **8a** there is disappearance of the 235 nm diameter objects due to **8a** and there is an increase in diameter for the proteins of between 5.2 and 7.5 nm, but with no objects of greater diameter observed, and no change in signal intensity. This implies that the interaction between **8a** and serum albumins does not cause precipitation.

The size increase for the protein is in accord with a monolayer of molecules of **8a** interacting with the protein. This is in fact similar to the previously observed behaviour for the interaction of bovine serum albumin and solid lipid nanoparticles formed by amphiphilic calix[4]arenes.[41] It should be noted that the information from dynamic light scattering does not allow either the determination of the stoichiometry of the complexation process nor determination of the association constants.

Haemolytic properties

The haemolytic properties of the *para*-octanoylcalix[8]arene derivatives are summarised in Table 4 below. A positive haemolytic effect is defined as that causing at least 5% lysis of the cell, hence only molecule **7a** can be designated as having haemolytic toxicity in the concentration range studied.

Table 4 present results of the haemolytic test. Assays were performed on one blood pool, at concentrations descending from 200 to 6.25 μg mL^{-1}. Results are expressed as percentage of haemolysis in comparison of the positive control, subtracting negative control.

Within the limits of experimental error only compound **7a**, *i.e.* with *O*-butoxyethyl ester functions, at concentrations between 25 μg mL^{-1} and 200 μg mL^{-1} and the parent compound **2a**

Table 4 Haemolysis percentage of molecules in comparison to the positive control

	Concentration as μg mL^{-1} of the total blood sample					
Molecule	200	100	50	25	12.5	6.25
2a	9.2	4.9	3.4	4.9	4.4	−0.3
3a	2.3	6.2	7.5	3.7	−3.0	−6.9
4a	3.1	2.0	3.0	4.8	−0.9	−6.5
6a	1.3	4.4	3.2	9.0	−4.1	−3.5
7a	9.7	6.6	7.4	5.9	−1.7	4.0
8a	4.9	0.9	5.1	7.3	2.7	−2.1

Table 3 Diameters of objects as measured by dynamic light scattering

	Observed Diameters/nm						
Protein	None	Bovine serum albumin	Horse SA	Rabbit SA	Goat SA	Chicken A	Lysozyme
Absence of **8a**	—	4.8	4.7	6.6	3.5	4.5	2.6
Presence of **8a**	235	11.1	9.9	12.5	11	13	46

at a concentration of 200 μg mL^{-1} show small but significant haemolytic properties. It is interesting to note that in terms of behaviour at the air–water interface **7a** shows significant behaviour with regard to the other compounds, with observation of a phase change between expanded liquid and condensed liquid phases, which may lead to differences in the membrane incorporation of this molecule and hence its small but significant haemolytic behaviour.

Evidently, the low aqueous solubility of all but **8a** has prevented analysis above μM concentrations. Thus the haemolytic effects for **7a** are significant, being 10^5 times higher than *para*-sulfonatocalix[8]arene[42] and approximately 10^3 times higher than those for β-cyclodextrin.[43]

Anticoagulant activity

The anticoagulant behaviour of a molecule is defined as its capacity to slow or stop the induced coagulation of blood. By convention molecules which cause blood coagulation times of more than 400 s are defined as giving rise to complete blocking of the coagulation process.

In Fig. 6 are given the anticoagulant properties of the molecules of series a, *i.e.* the *para*-octanoylcalix[8]arene derivatives, at concentrations of 20 μM and 100 μM respectively. The derivatives **8a** and **9a** both show total anticoagulant activity at concentrations of 100 μM as denoted by a relative percentage greater than 500%. **8a** shows clear activity at a concentration of 20 μM, with a relative coagulation time of 250%. These values are lower than those observed for heparin, which is active in nanomolar concentrations, however the activity is at least equal to that of the most potent calixarene derivative *para*-sulfonatocalix[8]arene monomethoxycarboxylate.[30]

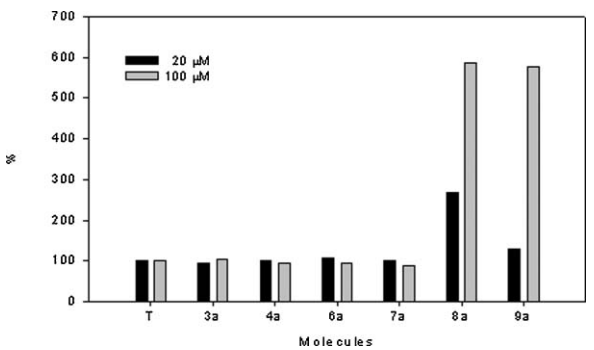


Fig. 6 Anticoagulant behaviour of compounds of the series of compounds derived from *para*-octanoylcalix[8]arene as measured by calcium dependent APTT times and given as percentage compared to 100% for the blank.

Experimental

General

Calix[8]arene,[44] **1**, and the *para*-acyl calix[8]arenes, **2a** and **2b**,[32] were synthesised as per the literature. Alkylation substrates, were purchased from Sigma-Aldrich, NaH 60% from Acros Organics, and used without further purification. All solvents were distilled, under a nitrogen atmosphere, over the appropriate drying agent immediately prior to use. All reactions were carried out under nitrogen.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXP 300 instrument operating at 500 MHz and 125 MHz respectively. The chemical shifts are reported from an internal tetramethylsilane standard.

Infra-red (IR) spectra were recorded on a Universal ATR in the range 4000–650 cm^{-1} as neat solids and are reported in cm^{-1}.

The melting point determinations were performed on a Beotius apparatus and are uncorrected.

Surface pressure isotherm measurements were carried out on a NIMA 6010 trough on Milli-Q quality water, with resistivity >18 MOhm, with the deposited molar quantity in the range 4×10^{-8}–1.6×10^{-8} mol, so as to generate isotherms with areas of 500 $Å^2$ prior to compression. The system was equilibrated for 30 minutes prior to compression, compression speed was 25 cm^2 min^{-1}. Deposition solvent was chloroform.

BAM images were collected using a Nano-Film Mini-BAM. Dynamic light scattering measurements were carried out on a Malvern Nanosizer.

Synthesis

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakis-4-cyanopropoxycalix[8]arene 3a. *para*-Octanoylcalix[8]-arene (1 g, 1 equiv.), 4-bromobutyronitrile (0.65 mL, 12 equiv.), potassium carbonate (0.9 g, 12 equiv.), and KI (0.1 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (2 × 30 mL) and dried under anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **3a**: ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 16H, *H*mAr), 4.06 (s, 16H, Ar–CH_2–Ar), 3.77 (t, 16H, Ar–O–CH_2, $J_{H-H} = 6.5$ Hz), 2.75 (t, 16H, –CH_2–CO, $J_{H-H} = 7.2$ Hz), 2.30 (t, 16H, –CH_2–CN, $J_{H-H} = 6.5$ Hz), 1.85 (m, 16H, –CH_2–CH_2–CH_2–), 1.60 (m, 16H, CH_2–CH_2–CO), 1.26 (m, 64 H, (–CH_2)4–), 0.85 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.6 (8C, C=O), 158.8 (8C, C_{ipso}), 133.9 (8C, C_{para}), 133.5 (16C, C_{ortho}), 129.7 (16C, C_{meta}), 119.1 (8C, CN), 71.0 (8C, ArO*C*H_2), 38.7 (8C, *C*H_2CO), 31.5–22.8 (48C, 7 × CH_2), 14.3 (8C, *C*H_3), 13.99 ppm (8C, –*C*H_2–CN). IR ν_{max} (neat): 2927 (CH_3), 2248 (CN), 1678 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2417.2 [**3a** + Na]$^+$, 2433.2 [**3a** + K]$^+$, mp = 140 °C, $R_f = 0.23$ ($CHCl_3$), yield = 72%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakismethoxyethoxycalix[8]arene 4a. *para*-Octanoylcalix[8]-arene (1 g, 1 equiv.), 2-bromoethyl methyl ether (0.6 mL, 12 equiv.), potassium carbonate (0.9 g, 12 equiv.), and KI (0.1 g, 1.1 equiv.) were combined and refluxed in acetone. After 48 h, the same quantities of potassium carbonate and 2-bromoethyl methyl ether were added to the solution and this was stirred and refluxed for 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **4a**: ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 16H, *H*mAr), 4.06 (s, 16H, Ar–CH_2–Ar), 3.68 (t, 16H, Ar–O–CH_2, $J_{H-H} = 6.6$ Hz),

3.37 (t, 16H, –CH_2–O–CH_3, J_{H-H} = 6.6 Hz), 3.26 (s, 24H, O–CH_3), 2.79 (s, 16H, –CH_2–CO, J_{H-H} = 7.1 Hz), 1.60 (m, 16H, CH_2–CH_2–CO), 1.27 (m, 64H, (–CH_2)4–), 0.89 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 199.4 (8C, C=O), 159.2 (8C, C_{ipso}), 134.5 (8C, C_{para}), 133.1 (16C, C_{ortho}), 129.8 (16C, C_{meta}), 72.6 (8C, $ArOCH_2$), 71.3 (8C, CH_2OCH_3), 58.7 (8C, OCH_3), 38.4 (8C, CH_2CO), 31.8–22.7 (48C, 6 × CH_2), 14.2 ppm (8C, CH_3). IR ν_{max} (neat): 2925 (CH_3), 1678 (Ar–C=O), 1045–1026 cm^{-1} (C–O). MS (MALDI-TOF): 2345.5 [**4a** + Na]$^+$, 2361.5 [**4a** + K]$^+$, mp = 60 °C, R_f = 0.24 ($CHCl_3$), yield = 62%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakismethoxydiethoxycalix[8]arene 5a. *para*-Octanoylcalix[8]-arene (1 g, 1 equiv.), 1-bromo-2-(2-methoxyethoxy)ethane (0.9 mL, 12 equiv.), potassium carbonate (0.9 g, 12 equiv.), and KI (0.1 g, 1.1 equiv.) were combined and refluxed in acetone. After 48 h, the same quantities of potassium carbonate and 1-bromo-2-(2-methoxyethoxy)ethane were added to the solution and this was stirred and refluxed for 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **5a**: ^{1}H NMR ($CDCl_3$) δ 7.64 (s, 16H, *H*mAr), 4.07 (s, 16H, Ar–CH_2–Ar), 3.79 (m, 16H, Ar–O–CH_2), 3.61 (m, 16H, –CH_2–CH_2–O–), 3.47 (m, 16H, CH_2–O–CH_2), 3.35 (m, 16H, –CH_2–O–CH_3), 3.28 (s, 24H, O–CH_3), 2.75 (t, 16H, –CH_2–CO, J_{H-H} = 7.2 Hz), 1.58 (m, 16H, –CH_2–CH_2–CO), 1.26 (m, 64H, (–CH_2)4–), 0.86 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 199.3 (8C, C=O), 159.3 (8C, C_{ipso}), 134.3 (8C, C_{para}), 133.0 (16C, C_{ortho}), 129.6 (16C, C_{meta}), 72.7 (8C, $ArOCH_2$), 71.8 (8C, $ArOCH_2CH_2$), 70.5 (8C, OCH_2CH_2O), 70.0 (8C, OCH_2CH_2O), 58.8 (8C, OCH_3), 38.4 (8C, CH_2CO), 32.0–22.4 (48C, 6 × CH_2), 14.2 ppm (8C, CH_3). IR ν_{max} (neat): 2924 (CH_3), 1679 (Ar–C=O), 1043 cm^{-1} (C–O). MS (MALDI-TOF): 2698.7 [**5a** + Na]$^+$, 2714.7 [**5a** + K]$^+$, mp = 75 °C, R_f = 0.65 ($CHCl_3$–MeOH 95 : 05), yield = 65%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakisethoxycarbonylmethoxycalix[8]arene 6a. *para*-Octanoylcalix[8]arene (1 g, 1 equiv.), ethyl bromoacetate (0.7 mL, 12 equiv.), potassium carbonate (0.9 g, 12 equiv.), and KI (0.1 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL) washed with water (2 × 30 mL) and dried under anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **6a**: ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 16H, *H*mAr), 4.27 (s, 16H, Ar–O–CH_2–), 4.13 (s, 16H, Ar–CH_2–Ar), 3.93 (q, 16H, O–CH_2–CH_3), 2.79 (t, 16H, –CH_2CO, J_{H-H} = 7.2 Hz), 1.59 (m, 16H, –CH_2–CH_2–CO), 1.26 (m, 64H, (–CH_2)4–), 1.03 (t, 24H, O–CH_2–CH_3, J_{H-H} = 6.9 Hz), 0.86 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 199.3 (8C, C=O), 168.4 (8C, *C*OO), 159.1 (8C, C_{ipso}), 133.6 (8C, C_{para}), 133.2 (16C, C_{ortho}), 129.8 (16C, C_{meta}), 69.9 (8C, Ar–O–CH_2), 61.2 (8C, O–CH_2–CH_3), 38.6 (8C, CH_2CO), 31.9–22.6 (48C, 6 × CH_2), 14.3 (8C, CH_3), 14.1 ppm (8C, CH_3). IR ν_{max} (neat): 2926 (CH_3), 1750 (C=O ester), 1678 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2570.5 [**6a** + Na]$^+$, 2586.5 [**6a** + K]$^+$, mp = 110 °C, R_f = 0.28 ($CHCl_3$), yield = 74%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakisethoxycarbonylpropoxycalix[8]arene 7a. *para*-Octanoylcalix[8]arene (1 g, 1 equiv.), ethyl bromobutyrate (0.9 mL, 12 equiv.), potassium carbonate (0.9 g, 12 equiv.), and KI (0.1 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (2 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **7a**: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 16H, *H*mAr), 4.05 (s, 16H, Ar–CH_2–Ar), 3.93 (q, 16H, O–CH_2–CH_3), 3.73 (t, 16H, Ar–O–CH_2, J_{H-H} = 6.7 Hz), 2.74 (t, 16H, –CH_2CO, J_{H-H} = 7.1 Hz), 2.33 (t, 16H, –CH_2–COOEt, J_{H-H} = 6.4 Hz), 1.93 (m, 16H, –CH_2–CH_2–CH_2–), 1.58 (m, 16H, –CH_2–CH_2–CO), 1.26 (m, 64H, (–CH_2)4–), 1.03 (t, 24H, O–CH_2–CH_3, J_{H-H} = 7.0 Hz), 0.85 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 199.2 (8C, C=O), 172.9 (8C, *C*OO), 159.3 (8C, C_{ipso}), 134.0 (8C, C_{para}), 133.2 (16C, C_{ortho}), 129.5 (16C, C_{meta}), 72.5 (8C, Ar–O–CH_2), 60.4 (8C, O–CH_2–CH_3), 38.5 (8C, CH_2CO), 32.1–22.9 (48C, 7 × CH_2), 14.3 (8C, CH_3), 14.2 ppm (8C, CH_3). IR ν_{max} (neat): 2927 (CH_3), 1731 (C=O ester), 1680 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2794.5 [**7a** + Na]$^+$, 2811.1 [**7a** + K]$^+$, mp = 120 °C, R_f = 0.29 ($CHCl_3$), yield = 78%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakissulfonatobutoxycalix[8]arene 8a. Under nitrogen atmosphere *para*-octanoylcalix[8]arene (1 g, 1 equiv.) was dissolved in 50 mL of freshly distilled THF and an excess of NaH (250 mg) was added. The mixture was stirred and refluxed and after 20 min 1,4-butane sultone (10 equiv.) was added. The introduction of NaH and 1,4-butane sultone was repeated 5 times during 2 days allowing total substitution of the hydroxyl groups and precipitation from the solution. The precipitate was filtered and washed with THF and MeOH.

Compound **8a**: ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 16H, *H*mAr), 4.07 (s, 16H, Ar–CH_2–Ar), 3.72 (t, 16H, Ar–O–CH_2), 2.97 (s, 16H, S–CH_2), 2.75 (m, 16H, –CH_2–CO), 1.64–1.58 (m, 40H, CH_2–CH_2–CO, CH_2–CH_2–CH_2–S), 1.20 (m, 64H, (–CH_2)4–), 0.82 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 199.2 (8C, C=O), 158.8 (8C, C_{ipso}), 134.1 (8C, C_{para}), 133.2 (16C, C_{ortho}), 129.7 (16C, C_{meta}), 72.4 (8C, $ArOCH_2$), 51.1 (8C, S–CH_2), 38.7 (8C, CH_2CO), 31.9–21.3 (48C, 8 × CH_2), 13.9 ppm (8C, CH_3). IR ν_{max} (neat): 3464 (OH), 2926 (CH_3), 1678 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2546.8 [**8a** − H]$^-$, mp >250 °, yield = 56%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakis-2-carboxymethoxycalix[8]arene 9a. **6a** (1 g, 1 equiv.) was added to 50 mL of a solution of KOH (10%) in ethanol–water (70 : 30) during 24 h. The ethanol was removed under reduced pressure. The compound was precipitated with a solution of HCl 2 M (60 mL) and filtered. The resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **9a**: ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 16H, *H*mAr), 4.21 (s, 16H, Ar–O–CH_2–), 4.09 (s, 16H, Ar–CH_2–Ar), 2.80 (t, 16H, –CH_2CO, $J_{H-H} = 7.1$ Hz), 1.61 (m, 16H, –CH_2–CH_2–CO), 1.26 (m, 64H, (–CH_2)4–), 0.85 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.3 (8C, C=O), 171.2 (8C, *C*OO), 158.5 (8C, C_{ipso}), 133.9 (8C, C_{para}), 133.5 (16C, C_{ortho}), 129.1 (16C, C_{meta}), 69.8 (8C, Ar–O–*C*H_2), 38.9 (8C, *C*H_2CO), 31.9–22.9 (48C, 6 × CH_2), 14.3 (8C, *C*H_3). IR ν_{max} (neat): 3201 (OH acid), 2925 (CH_3), 1729 (C=O acid), 1679 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2345.2 [**9a** + Na]$^+$, 2361.2 [**9a** + K]$^+$, mp = 145 °C, $R_f = 0.81$ ($CHCl_3$–MeOH 80 : 20), yield = 79%.

5,11,17,23,29,35,41,47-Octaoctanoyl-49,50,51,52,53,54,55,56-octakis-4-carboxypropoxycalix[8]arene 10a. **7a** (1 g, 1 equiv.) was added to 50 mL of a solution of KOH (10%) in ethanol–water (70 : 30) during 24 h. The ethanol was removed under reduced pressure. The compound was precipitated with a solution of HCl 2 M (60 mL) and filtered. The resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **10a**: ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 16H, *H*mAr), 4.06 (s, 16H, Ar–CH_2–Ar), 3.62 (t, 16H, Ar–O–CH_2–, $J_{H-H} = 6.6$ Hz), 2.76 (t, 16H, –CH_2–CO, $J_{H-H} = 7.1$ Hz), 2.37 (t, 16H, –CH_2–CH_2–COO, $J_{H-H} = 6.4$ Hz), 1.87 (m, 16H, –CH_2–CH_2–CH_2–), 1.59 (m, 16H, –CH_2–CH_2–CO), 1.24 (m, 64H, (–CH_2)4–), 0.85 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.8 (8C, C=O), 178.8 (8C, *C*OO), 159.3 (8C, C_{ipso}), 133.8 (8C, C_{para}), 133.2 (16C, C_{ortho}), 129.5 (16C, C_{meta}), 72.5 (8C, Ar–O–*C*H_2), 38.7 (8C, *C*H_2CO), 31.7–22.3 (56C, 7 × CH_2), 14.3 (8C, *C*H_3). IR ν_{max} (neat): 3205 (OH acid), 2924 (CH_3), 1707 (C=O acid), 1679 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 2569.5 [**10a** + Na]$^+$, 2585.5 [**10a** + K]$^+$, mp = 190 °C, $R_f = 0.78$ ($CHCl_3$–MeOH 80 : 20), yield = 69%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakis-4-cyanopropoxycalix[8]arene 3b. *para*-Palmitoylcalix[8]arene (1 g, 1 equiv.), 4-bromobutyronotrile (0.45 mL, 12 equiv.), potassium carbonate (0.6 g, 12 equiv.), and KI (0.06 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (2 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **3b**: ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 16H, *H*mAr), 4.05 (s, 16H, Ar–CH_2–Ar), 3.75 (t, 16H, Ar–O–CH_2, $J_{H-H} = 6.6$ Hz), 2.75 (t, 16H, –CH_2–CO, $J_{H-H} = 7.1$ Hz), 2.29 (t, 16H, –CH_2–CN, $J_{H-H} = 6.6$ Hz), 1.85 (m, 16H, –CH_2–CH_2–CH_2–), 1.60 (m, 16H, CH_2–CH_2–CO), 1.26 (m, 192H, (–CH_2)12–), 0.88 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.6 (8C, C=O), 158.8 (8C, C_{ipso}), 133.9 (8C, C_{para}), 133.6 (16C, C_{ortho}), 129.7 (16C, C_{meta}), 119.4 (8C, CN), 71.0 (8C, Ar–O–*C*H_2), 38.4 (8C, *C*H_2CO), 32.3–22.9 (120C, 15 × CH_2), 14.5 (8C, *C*H_3), 13.9 (8C, –*C*H_2–CN). IR ν_{max} (neat): 2917 (CH_3), 2247 (CN), 1682 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 3315.0 [**3b** + Na]$^+$, 3331.0 [**3b** + K]$^+$, mp = 145 °C, $R_f = 0.25$ ($CHCl_3$), yield = 71%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakismethoxyethoxycalix[8]arene 4b. *para*-Palmitoylcalix[8]arene (1 g, 1 equiv.), 2-bromoethyl methyl ether (0.4 mL, 12 equiv.), potassium carbonate (0.6 g, 12 equiv.), and KI (0.06 g, 1.1 equiv.) were combined and refluxed in acetone. After 48 h, the same quantities of potassium carbonate and 2-bromoethyl methyl ether were added to the solution and this was stirred and refluxed for 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried under anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **4b**: ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 16H, *H*mAr), 4.09 (s, 16H, Ar–CH_2–Ar), 3.68 (t, 16H, Ar–O–CH_2, $J_{H-H} = 6.6$ Hz), 3.39 (t, 16H, CH_2–O–CH_3, $J_{H-H} = 6.6$ Hz), 3.22 (s, 24H, –CH_2–O–CH_3), 2.76 (t, 16H, –CH_2–CO, $J_{H-H} = 6.6$ Hz), 1.59 (m, 16H, CH_2–CH_2–CO), 1.26 (m, 192H, (–CH_2)12–), 0.87 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.4 (8C, C=O), 159.3 (8C, C_{ipso}), 134.6 (8C, C_{para}), 133.2 (16C, C_{ortho}), 129.8 (16C, C_{meta}), 72.7 (8C, Ar–O–*C*H_2), 71.4 (8C, CH_2OCH_3), 58.8 (8C, OCH_3), 38.4 (8C, *C*H_2CO), 32.1–22.9 (112C, 14 × CH_2), 14.5 ppm (8C, *C*H_3). IR ν_{max} (neat): 2918 (CH_3), 1680 (Ar–C=O), 1046–1027 cm^{-1} (C–O). MS (MALDI-TOF): 3243.2 [**4b** + Na]$^+$, 3259.3 [**4b** + K]$^+$, mp = 65 °C, $R_f = 0.33$ ($CHCl_3$), yield = 72%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakismethoxydiethoxycalix[8]arene 5b. *para*-Palmitoylcalix[8]arene (1 g, 1 equiv.), 1-bromo-2-(2-methoxyethoxy)ethane (0.58 mL, 12 equiv.), potassium carbonate (0.6 g, 12 equiv.), and KI (0.06 g, 1.1 equiv.) were combined and refluxed in acetone. After 48 h, the same quantities of potassium carbonate and 1-bromo-2-(2-methoxyethoxy)ethane were added to the solution and this was stirred and refluxed for 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **5b**: ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 16H, *H*mAr), 4.08 (s, 16H, Ar–CH_2–Ar), 3.77 (m, 16H, Ar–O–CH_2), 3.60 (m, 16H, –CH_2–CH_2–O–), 3.47 (m, 16H, CH_2–O–CH_2), 3.38 (m, –CH_2–O–CH_3), 3.27 (s, 24H, O–CH_3), 2.73 (t, 16H, –CH_2–CO, $J_{H-H} = 7.2$ Hz), 1.57 (m, 16H, CH_2–CH_2–CO), 1.25 (m, 192H, (–CH_2)12–), 0.85 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.2 (8C, C=O), 159.6 (8C, C_{ipso}), 134.3 (8C, C_{para}), 133.0 (16C, C_{ortho}), 129.7 (16C, C_{meta}), 72.1 (8C, Ar–O–*C*H_2), 70.7 (8C, ArOCH_2*C*H_2), 70.3 (8C, O*C*H_2CH_2O), 69.5 (8C, OCH_2*C*H_2O), 59.3 (8C, OCH_3), 38.4 (8C, *C*H_2CO), 32.3–24.8 (112C, 14 × CH_2), 14.3 ppm (8C, *C*H_3). IR ν_{max} (neat): 2921 (CH_3), 1679 (Ar–C=O), 1045 cm^{-1} (C–O). MS (MALDI-TOF): 3597.7 [**5b** + Na]$^+$, mp = 81 °C, $R_f = 0.61$ ($CHCl_3$–MeOH 95 : 05), yield = 70%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakisethoxycarbonylmethoxycalix[8]arene 6b. *para*-Palmitoylcalix[8]arene (1 g, 1 equiv.), ethyl bromoacetate (0.5 mL, 12 equiv.), potassium carbonate (0.6 g, 12 equiv.), and KI (0.06 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL)

washed with water (2 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **6b**: ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 16H, *H*mAr), 4.27 (s, 16H, Ar–O–CH_2), 4.14 (s, 16H, Ar–CH_2–Ar), 3.95 (q, 16H, O–CH_2–CH_3), 2.79 (t, 16H, –CH_2–CO, $J_{H-H} = 7.2$ Hz), 1.59 (m, 16H, CH_2–CH_2–CO), 1.27 (m, 192H, (–CH_2)12–), 1.03 (t, O–CH_2–CH_3, $J_{H-H} = 6.8$ Hz), 0.88 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.6$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.3 (8C, C=O), 168.4 (8C, *C*OO), 159.1 (8C, C_{ipso}), 134.2 (8C, C_{para}), 133.6 (16C, C_{ortho}), 129.8 (16C, C_{meta}), 69.9 (8C, Ar–O–*C*H_2), 61.2 (8C, O–*C*H_2–CH_3), 38.4 (8C, *C*H_2CO), 31.8–22.8 (112C, 14 × CH_2), 14.5 (8C, *C*H_3), 14.1 ppm (8C, *C*H_3). IR ν_{max} (neat): 2916 (CH_3), 1749 (C=O ester), 1687 cm^{-1} (Ar–CO). MS (MALDI-TOF): 3467.8 [**6b** + Na]$^+$, 3525.8 [**6b** + K]$^+$, mp = 115 °C, $R_f = 0.45$ ($CHCl_3$), yield = 75%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakisethoxycarbonylpropoxycalix[8]arene 7b. *para*-Palmitoylcalix[8]arene (1 g, 1 equiv.), ethyl bromobutyrate (0.62 mL, 12 equiv.), potassium carbonate (0.6 g, 12 equiv.), and KI (0.06 g, 1.1 equiv.) were combined and refluxed in acetone during 72 h. The acetone was removed under reduced pressure and the resultant compound was solubilised in chloroform (30 mL), washed with water (2 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **7b**: ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 16H, *H*mAr), 4.04 (s, 16H, Ar–CH_2–Ar), 3.93 (q, 16H, –O–CH_2–CH_3), 3.73 (t, 16H, Ar–O–CH_2–, J_{H-H} = 6.6 Hz), 2.73 (t, 16H, CH_2–CO, J_{H-H} = 7.2 Hz), 2.35 (t, 16H, –CH_2–COOEt, $J_{H-H} = 6.5$ Hz), 1.93 (m, 16H, –CH_2–CH_2–CH_2–), 1.57 (m, 16H, –CH_2–CH_2–CO), 1.28 (m, 192H, (–CH_2)12–), 1.03 (t, 24H, O–CH_2–CH_3, $J_{H-H} = 7.0$ Hz), 0.89 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.6$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.5 (8C, C=O), 171.3 (8C, *C*OO), 159.5 (8C, C_{ipso}), 134.2 (8C, C_{para}), 133.1 (16C, C_{ortho}), 129.7 (16C, C_{meta}), 71.9 (8C, Ar–O–*C*H_2), 60.2 (8C, O–*C*H_2–CH_3), 38.1 (8C, *C*H_2CO), 32.2–22.8 (120C, 15 × CH_2), 14.5 (8C, *C*H_3), 14.1 ppm (8C, *C*H_3). IR ν_{max} (neat): 2925 (CH_3), 1732 (C=O ester), 1685 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 3692.4 [**7b** + Na]$^+$, 3708.4 [**7b** + K] $^+$, mp = 125 °C, R_f = 0.38 ($CHCl_3$), yield = 73%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakis-2-carboxymethoxycalix[8]arene 9b. **6b** (1 g, 1 equiv.) was added in 50 mL of a solution of KOH (10%) in ethanol–water (70 : 30) during 24 h. The ethanol was removed under reduced pressure. Compound was precipitated with a solution of HCl 2 M (60 mL) and filtered. The resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **9b**: ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 16H, *H*mAr), 4.26 (s, 16H, Ar–O–CH_2–), 4.11 (s, 16H, Ar–CH_2–Ar), 2.80 (t, 16H, –CH_2–CO, $J_{H-H} = 7.2$ Hz), 1.61 (m, 16H, –CH_2–CH_2–CO), 1.26 (m, 192H, (–CH_2)12–), 0.85 ppm (t, 24H, CH_3–CH_2, J_{H-H} = 6.6 Hz). ^{13}C NMR ($CDCl_3$) δ 199.3 (8C, C=O), 170.4 (8C, *C*OO), 158.3 (8C, C_{ipso}), 134.5 (8C, C_{para}), 133.9 (16C, C_{ortho}), 129.9 (16C, C_{meta}), 69.8 (8C, Ar–O–*C*H_2), 38.7 (8C, *C*H_2CO), 32.1–22.1 (112C, 14 × CH_2), 14.1 ppm (8C, *C*H_3). IR ν_{max} (neat): 3197 (OH acid), 2919 (CH_3), 1738 (C=O acid), 1683 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 3219.9 [**9b** − H]$^-$, mp = 150 °C, $R_f = 0.78$ ($CHCl_3$–MeOH 80 : 20), yield = 61%.

5,11,17,23,29,35,41,47-Octahexadecanoyl-49,50,51,52,53,54,55,56-octakis-4-carboxypropoxycalix[8]arene 10b. **7b** (1 g, 1 equiv.) was added to 50 mL of a solution of KOH (10%) in ethanol–water (70 : 30) during 24 h. The ethanol was removed under reduced pressure. Compound was precipitated with a solution of HCl 2 M (60 mL) and filtered. The resultant compound was solubilised in chloroform (30 mL), washed with water (4 × 30 mL) and dried over anhydrous $MgSO_4$. The chloroform was removed under reduced pressure to give a volume of 5 mL and the product was precipitated in methanol.

Compound **10b**: ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 16H, *H*mAr), 4.08 (s, 16H, Ar–CH_2–Ar), 3.65 (t, 16H, Ar–O–CH_2–, $J_{H-H} = 6.6$ Hz), 2.73 (t, 16H, –CH_2–CO, $J_{H-H} = 7.2$ Hz), 2.38 (t, 16H, –CH_2–CH_2–CH_2–O, $J_{H-H} = 6.7$ Hz), 1.85 (m, 16H, –CH_2–CH_2–CH_2–), 1.57 (m, 16H, –CH_2–CH_2–CO), 1.24 (m, 192H, (–CH_2)12–), 0.83 ppm (t, 24H, CH_3–CH_2, $J_{H-H} = 6.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 199.4 (8C, C=O), 179.6 (8C, *C*OO), 158.5 (8C, C_{ipso}), 134.1 (8C, C_{para}), 133.8 (16C, C_{ortho}), 129.3 (16C, C_{meta}), 71.3 (8C, Ar–O–*C*H_2), 38.4 (8C, *C*H_2CO), 31.5–22.5 (120C, 15 × CH_2), 14.4 ppm (8C, *C*H_3). IR ν_{max} (neat): 3194 (OH acid), 2916 (CH_3), 1704 (C=O acid), 1683 cm^{-1} (Ar–C=O). MS (MALDI-TOF): 3466.7 [**10b** + Na]$^+$, 3482.7 [**10b** + K]$^+$, mp = 195 °C, $R_f = 0.8$ ($CHCl_3$–MeOH 80 : 20), yield = 61%.

Dymanic light scattering

Particle sizes were measured in water and PBS using a Malvern Nanosizer with 45 µL cells. Ten runs were collected for each measurement, three measurements were averaged, all measurements showed deviation of less than 5%. Both standard and multimodal analysis were carried out on the results.

Haemolytic properties

The *in vitro* haemolytic test is derived from the method described by Fairbanks *et al.*,[45] particularly the method of Harboe. The principle of the test consists in the spectrophotometric measurement of the haemoglobin released by lysis of red blood cells. This test allows the measurement of the ability of the tested molecules to break the membranes of red blood cells and then to estimate compound toxicity against red blood cells. Red blood cells are extracted by centrifugation of total blood samples and incubated at 37 °C for half an hour with different concentrations of the molecules. Where there is sufficient aqueous solubility the molecules are dissolved in PBS, and where aqueous solubility is not sufficient the molecules are dissolved in DMSO which is added to form no more than 5% of the total volume. Controls are red blood cells alone in the relevant solvent system for the negative control, and red blood cells lysed by cold MilliQ water for positive control. The haemoglobin released from lysed cells is measured spectrometrically at 540 nm. To calculate the ratio of haemolysis, the positive control is considered as 100% haemolysis and the negative control as 0% haemolysis.

Coagulation time measurements

Activated partial thromboplastin time (APTT). The time of plasma coagulation is measured using an optical and mechanical Coagulometer 2 Channel COA-Titer 2 apparatus, from CGA Strummenti Scientifici S.P.A. (Firenze, Italy).

The *para*-acyl-calix[8]arene derivatives were dissolved in DMSO at a concentration of 10 mg mL^{-1} and then rediluted in aqueous isotonic NaCl (154 mM) to give the desired concentrations, 1 g L^{-1} and 5 g L^{-1}. To 100 μL of fresh human pool blood plasma and 100 μL of kaolin solution were added 20 μL aliquots of the *para*-acyl-calix[8]arene solutions and the mixture was incubated at 37 °C during 3 minutes. At this time factor XII is autoactivated and converts prekallikrein to kallikrein, which then activates factor XII to factor XIIa. The blood coagulation cascade resulting in thrombin generation and conversion of fibrinogen to fibrin was started by the addition of 100 μL of a 0.025 M solution of $CaCl_2$. The time of coagulation is measured at least twice to confirm reproducibility.

Conclusions

Two novel series of derivatives of the *para*-acyl-calix[8]arenes have been synthesised generally in excellent yields. Study of the interfacial properties of the two series shows that the molecules having octyl chains as the *para*-function have a strong tendency to self-organise into 3D structures at the air–water interface while those having longer C16 chains tend to behave as a more classical amphiphilic molecule, forming apparent mono-layers. The biological properties of these compounds are interesting with a general lack of haemolytic toxicity, and anticoagulant activity for two of the anionic derivatives. The *O*-4-butylsulfonate derivative of *para*-octylcalix[8]arene, which shows aqueous solubility, has been demonstrated to be capable of complexing the serum albumin proteins of various animal sources.

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References

1 C. D. Gutsche, *Calixarenes Revisited*, Royal Society of Chemistry, Cambridge, 1998.
2 *Calixarenes 2001*, ed. Z. Asfari, V. Bohmer, J. Harrowfield and J. Vicens, Kluwer Academic Publisher, Dordrecht, 2001.
3 A. W. Coleman, A. N. Lazar and E. Da Silva, Calixarenes and their analogues: Cation Complexation in *Encyclopedia of Supramolecular Chemistry*, ed. J. L. Atwood and J. W. Steed, Taylor & Francis, New York, 2004, pp. 137–144.
4 M. H. Filby and J. W. Steed, *Coord. Chem. Rev.*, 2006, **250**, 3200–3218.
5 S. J. Dalgarno, P. K. Thallapally, L. J. Barbour and J. L. Atwood, *Chem. Soc. Rev.*, 2007, **36**, 236–245.
6 R. Cacciapaglia, A. Casnati, L. Mandolini, D. N. Reinhoudt, R. Salvio, A. Sartori and R. Ungaro, *J. Am. Chem. Soc.*, 2006, **128**, 12322–12330.
7 Y. Shi and H.-J. Schneider, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1797–1803.
8 E. Da Silva and A. W. Coleman, *Tetrahedron*, 2003, **59**, 7357–7364.
9 N. Douteau-Guevel, F. Perret, A. W. Coleman, J.-P. Morel and N. Morel-Desrosiers, *J. Chem. Soc., Perkin Trans. 2*, 2002, 524–532.
10 A. W. Coleman, F. Perret, S. Cecillon, A. Moussa, A. Martin, M. Dupin and H. Perron, *New J. Chem.*, 2007, **31**, 711–717.
11 A. W. Coleman, F. Perret, A. Moussa, M. Dupin, Y. Guo and H. Perron, *Top. Curr. Chem.*, 2007, **277**, 31–88.
12 M. J. Potrzebowski and S. Kazmierski, *Top. Curr. Chem.*, 2005, **246**, 91–140.
13 A. F. D. de Namor, R. M. Cleverley and M. L. Zapata-Ormachea, *Chem. Rev.*, 1998, **98**, 2495–2525.
14 G. S. Ananchenko, K. A. Udachin, A. Dubes, J. A. Ripmeester, T. Perrier and A. W. Coleman, *Angew. Chem., Int. Ed.*, 2006, **45**, 1585–1588.
15 E. Da Silva, C. Valmalle, M. Becchi, C.-Y. Cuilleron and A. W. Coleman, *J. Inclusion Phenom. Macrocyclic Chem.*, 2003, **46**, 65–69.
16 S. Kolusheva, R. Zadmard, T. Schrader and R. Jelinek, *J. Am. Chem. Soc.*, 2006, **128**, 13592–13598.
17 K. No and Y. Kim, *Bull. Korean Chem. Soc.*, 1988, **9**, 52–55.
18 S. Shinkai, T. Nagasaki, K. Iwamoto, A. Ikeda, G. X. He, T. Matsuda and M. Iwamoto, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 381–386.
19 P. Shahgaldian, A. W. Coleman and V. I. Kalchenko, *Tetrahedron Lett.*, 2001, **42**, 577–579.
20 P. Shahgaldian, M. Cesario, P. Goreloff and A. W. Coleman, *Chem. Commun.*, 2002, 326–327.
21 P. Shahgaldian and A. W. Coleman, *Langmuir*, 2001, **17**, 6851–6854.
22 P. Shahgaldian, E. Da Silva, A. W. Coleman, B. Rather and M. J. Zaworotko, *Int. J. Pharm.*, 2003, **253**, 23–38.
23 P. Shahgaldian, L. Quattrocchi, J. Gualbert, A. W. Coleman and P. Goreloff, *Eur. J. Pharm. Biopharm.*, 2003, **55**, 107–113.
24 P. Shahgaldian, J. Gualbert, K. Aissa and A. W. Coleman, *Eur. J. Pharm. Biopharm.*, 2003, **55**, 181–184.
25 G. S. Ananchenko, K. A. Udachin, M. Pojarova, S. Jebors, A. W. Coleman and J. A. Ripmeester, *Chem. Commun.*, 2007, 707–709.
26 G. S. Ananchenko, K. A. Udachin, J. A. Ripmeester, T. Perrier and A. W. Coleman, *Chem.–Eur. J.*, 2006, **12**, 2441–2447.
27 M. Pojarova, G. S. Ananchenko, K. A. Udachin, M. Daroszewska, F. Perret, A. W. Coleman and J. A. Ripmeester, *Chem. Mater.*, 2006, **18**, 5817–5819.
28 A. Dubes, I. L. Moudrakovski, P. Shahgaldian, A. W. Coleman, C. I. Ratcliffe and J. A. Ripmeester, *J. Am. Chem. Soc.*, 2004, **126**, 6236–6237.
29 A. Lazar, E. Da Silva, A. Navaza, C. Barbey and A. W. Coleman, *Chem. Commun.*, 2004, 2162–2163.
30 E. D. Silva, D. Ficheux and A. W. Coleman, *J. Inclusion Phenom. Macrocyclic Chem.*, 2005, **52**, 201–206.
31 M. Aoki, K. Nakashima, H. Kawabata, S. Tsutsui and S. Shinkai, *J. Chem. Soc., Perkin Trans. 2*, 1993, 347–354.
32 S. Jebors, G. S. Ananchenko, A. W. Coleman and J. A. Ripmeester, *Tetrahedron Lett.*, 2007, **48**, 5503–5506.
33 F. A. Loiseau, A. M. Hill and K. K. Hii, *Tetrahedron*, 2007, **63**, 9947–9959.
34 S. Shinkai, T. Arimura, K. Araki, H. Kawabata, H. Satoh, T. Tsubaki, O. Manabe and J. Sunamoto, *J. Chem. Soc., Perkin Trans. 1*, 1989, 2039–2045.
35 G. De Miguel, J. M. Pedrosa, M. T. Martin-Romero, E. Munoz, T. H. Richardson and L. Camacho, *J. Phys. Chem. B*, 2005, **109**, 3998–4006.
36 R. Castillo, S. Ramos, R. Cruz, M. Martinez, F. Lara and J. Ruiz-Garcia, *J. Phys. Chem.*, 1996, **100**, 709–713.
37 L. Dei, A. Casnati, P. Lo Nostro and P. Baglioni, *Langmuir*, 1995, **11**, 1268–1272.
38 L. Dei, P. LoNostro, G. Capuzzi and P. Baglioni, *Langmuir*, 1998, **14**, 4143–4147.
39 T. Mecca, G. M. L. Consoli, C. Geraci, R. La Spina and F. Cunsolo, *Org. Biomol. Chem.*, 2006, **4**, 3763–3768.
40 E. Da Silva, C. F. Rousseau, I. Zanella-Cleon, M. Becchi and A. W. Coleman, *J. Inclusion Phenom. Macrocyclic Chem.*, 2006, **54**, 53–59.
41 J. Gualbert, P. Shahgaldian and A. W. Coleman, *Int. J. Pharm.*, 2003, **257**, 69–73.
42 E. Da Silva, P. Shahgaldian and A. W. Coleman, *Int. J. Pharm.*, 2004, **273**, 57–62.
43 M. Bost, V. Laine, F. Pilard, A. Gadelle, J. Defaye and B. Perly, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1997, **29**, 57–63.
44 *Macrocycle Synthesis: A Practical Approach*, ed. D. Parker, OUP, Oxford, 1996.
45 V. F. Fairbanks, S. C. Ziesmer and P. C. O'Brien, *Clin. Chem.*, 1992, **38**, 132–140.