

Synthesis, Crystal Structure and Cytotoxicity Studies of *p*-Sulfonatocalix[4]arene Lanthanide Complexes

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A number of *p*-sulfonatocalix[4]arene complexes of the lanthanides (Tb, Gd, and Eu) have been prepared, some in the presence of tetraazamacrocycle 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A), and fully characterised. Crystal structure determinations reveal lanthanide coordination at the sulfonate group, bridging several calixarene units, giving coordination polymers. All complexes in this study have been determined to be relatively non-toxic using in vitro cell assays with CC₅₀ values in the range 30 – 170 μM.

Introduction

The calixarenes are a major class of macrocycle synthesised by the condensation reaction between para substituted phenols and formaldehyde. One disadvantage of these macrocycles is that unmodified, they are highly insoluble. However with modification it is possible to increase the solubility of these macrocycles. In order for these molecules to be utilised in a biological setting, they must be soluble in water and so in 1984

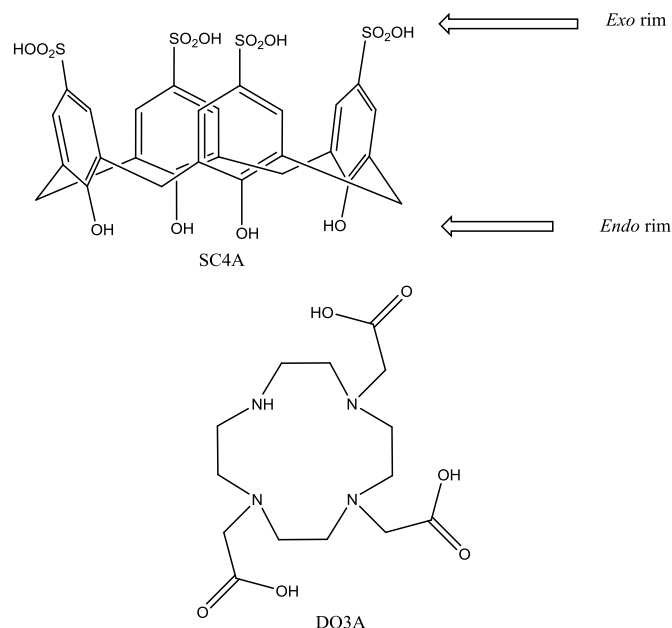


Fig. 1 The structures of SC4A showing the *exo* and *endo* rims, and DO3A.

the first reported water soluble calixarene was reported by Ungaro *et al.*¹ Also in 1984 the first calixarene derivative with sulfonate groups at the para positions of the phenolic rings was synthesised. *p*-Sulfonatocalix[*n*]arenes (*n* = 4–8, Fig. 1, SCnAs) are a class of highly water-soluble calixarene first reported by Shinkai *et al.*²

They are easily prepared by direct sulfonation of the *exo* rim of *p*-*tert*-butylcalix[4]arene providing sulfonic acid groups which allow them flexible inclusion/complexation properties.³ Previous studies have shown that SCnAs are biocompatible^{4,5}, in that they are both non-toxic and water-soluble, which makes them eligible for biological and pharmaceutical applications.^{6,7} For these reasons, we have selected SCnAs for the present study.

A considerable amount of work on SC4A lanthanide complexes has been carried out previously.^{8–14} Work by Atwood *et al.* details the characterisation of a series of new structural motifs combining trivalent lanthanide ions and SC4As, and describes structures consisting of bi-layers of SC4A molecules linked by lanthanide cations as well as complex supramolecular assemblies.¹⁵ These assemblies consist of crown ether molecules sat within the hydrophobic cavity created by two calixarene molecules.¹⁵ This type of encapsulation is not just restricted to crown ethers, many other cationic, anionic, and neutral molecules can reside within the cavities formed by SCnA molecules.^{16–22}

Further work by Dalgarno and Atwood, describes a coordination polymer formed with alternating dipyrindine/SC6As linked with lanthanide cations, which also consists of a bi-layer arrangement.²³ It was also demonstrated that by controlling the pH, structures where two SC4As envelop a crown ether molecule can be obtained.²⁴ It is noted that the majority of this previous work concentrates on crystal engineering with little or no biological studies being presented. The biological applications of calixarenes has recently been reviewed.²⁵

Another macrocycle often used for biological applications is 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A,

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shown **Fig. 1**.²⁶ Synthesised from the reaction between 1,4,7,10-tetraazacyclododecane (cyclen) and *t*-butylbromoacetate,²⁷ this particular macrocycle is an efficient chelator of di- and tri- valent cations due to its acetate arms, which present strong binding donor groups.²⁸ Derivatives of DO3A containing a bound gadolinium(III) ion have been utilised as magnetic resonance imaging (MRI) contrast agents due to the magnetic properties of the lanthanide. For example, a conjugated 2-(diphenylphosphoryl)-ethylidiphenylphosphonium cation DO3A derivative was reported that exhibited low cytotoxicity and high relaxivity.²⁹ Uptake studies revealed a remarkable affinity for tumour cells and *in vitro*, they showed a higher T_1 relaxivity measurement was observed compared to the clinical contrast agents Magnevist and Dotarem.²⁹ The low cytotoxicity shows that DO3A is an excellent chelate for Gd(III) MRI contrast agents. In 2013 an aniline benzothiazole conjugate has comparable R_1 relaxivity to Dotarem and has a specificity towards the MDA-MB-231 breast cancer cell line.³⁰ This shows that facile modification of the DO3A Chelate can allow for targeting to specific disease states.³¹

It has been shown that attaching tetraazamacrocyclic chelators to calixarene molecules leads to efficient contrast agents.³² For example Peters *et al.* have conducted research into the use of 1,4,7,10-tetra(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA) calixarene conjugates, that exhibit good relaxivities, as potential MRI contrast agents.³³ These molecules form aggregates which can also accommodate radionuclides to produce next generation multimodal imaging agents combining nuclear medicine diagnostics (PET/SPECT) with MRI.^{33,34}

Both calixarenes and tetraazamacrocyclics are used as metal chelators in the search for new biologically active metal complexes to combat disease. To the best of our knowledge, the cytotoxicity of the type of complex presented herein has not previously been reported. In particular, we present the synthesis and characterisation of SC4A lanthanide complexes and demonstrate their low cytotoxicity.

Results and Discussion

Synthesis and characterisation

p-Sulfonatocalix[4]arene and DO3A were prepared according to previously described methods.^{38,27}

Complexes **(1)**, **(4)**, and **(5)** were prepared by heating an aqueous solution of $\text{Ln}(\text{O}_2\text{C}_2\text{H}_3)_3$ and *p*-sulfonatocalix[4]arene for several hours and then allowing the solution to cool and evaporate slowly over the course of several days.

Complexes **(3)**, **(6)**, and **(7)** were prepared in a similar fashion with the addition of 1 eq. of DO3A into the aqueous reaction mixture.

Complexes **(8)**, **(9)**, and **(10)** were prepared in the same manner as **(3)**, **(6)**, and **(7)**, however the quantity of the lanthanide salt used was increased by 100% in an attempt to get a structure with a lanthanide ion coordinated to a DO3A chelator.

Complex **(2)** was prepared by heating an aqueous solution of Gd-DO3A and *p*-sulfonatocalix[4]arene for several hours and then allowing the solution to cool and evaporate slowly over the course of several days. **Table 1.** shows the molar ratios of each component used to synthesise each complex.

Complex	Ln salt (eq.)	SC4A (eq.)	DO3A (eq.)
(1)	2.2 (Tb)	1	0
(2)	1 (Gd)	1	0
(3)	1 (Eu)	1	1
(4)	2.2 (Gd)	1	0
(5)	2.2 (Eu)	1	0
(6)	1 (Tb)	1	1
(7)	1 (Gd)	1	1
(8)	2 (Eu)	1	1
(9)	2 (Gd)	1	1
(10)	2 (Tb)	1	1

Table 1 A summary of the molar equivalents of each component used to synthesise each component.

All complexes show lanthanide ions binding at the sulfonate groups of the SC4As and in most cases bridge two SC4As forming long coordination polymers. However the mass spectrometry results suggest that these chains of lanthanide bridged SC4As readily break apart in aqueous solution as much lower than expected molecular ion peaks are observed.

The structure of the ligand (**Fig. 2**) is comparable to a structure previously reported by Atwood *et al.* in 1988 (**Fig. 3**).³⁵ Atwood's structure shows an SC4A molecule with a methyl sulfate anion residing in the cavity of the SC4A, whereas our structure reveals a dichloromethane molecule residing in the cavity.

For complex **(1)** the SC4A molecules form two discrete structural motifs. Both these motifs are based on Tb ions bridging SC4A molecules in a way seen previously by Raston *et al.* (**Fig. 3**).²⁴ Both structures show SC4A molecules bridged by Ln ions where the coordination of the Ln ion is completed by water. Both structures also reveal a large number of unbound water molecules in their respective asymmetric units.

Atwood *et al.* published a structure very similar to complex **(2)** in 2001 (**Fig. 3**).³⁶ The hetero-bimetallic cage described by Atwood comprised of two SC4A molecules forming a dimer through Na bridges with each SC4A also coordinating to one Y ion. The coordination about each metallic centre is completed by water in both structures. Complex **(2)** is very similar in that two SC4A molecules form a dimer through Na bridges with each SC4A also coordinating to one Gd ion, rather than one Y ion.

Whilst we have not been able to obtain a crystallographic model in which all the intricate details are resolved for complex **(3)**, we have been able to extract chemically useful information from the data. One portion of the structure shows a DO3A molecule residing in the cavity of an SC4A molecule. This is not unlike the structure reported by Raston *et al.* in 2006³⁷ (**Fig. 3**), where a diprotonated diaza-12-crown-4 sits in the cavity of an SC4A molecule.

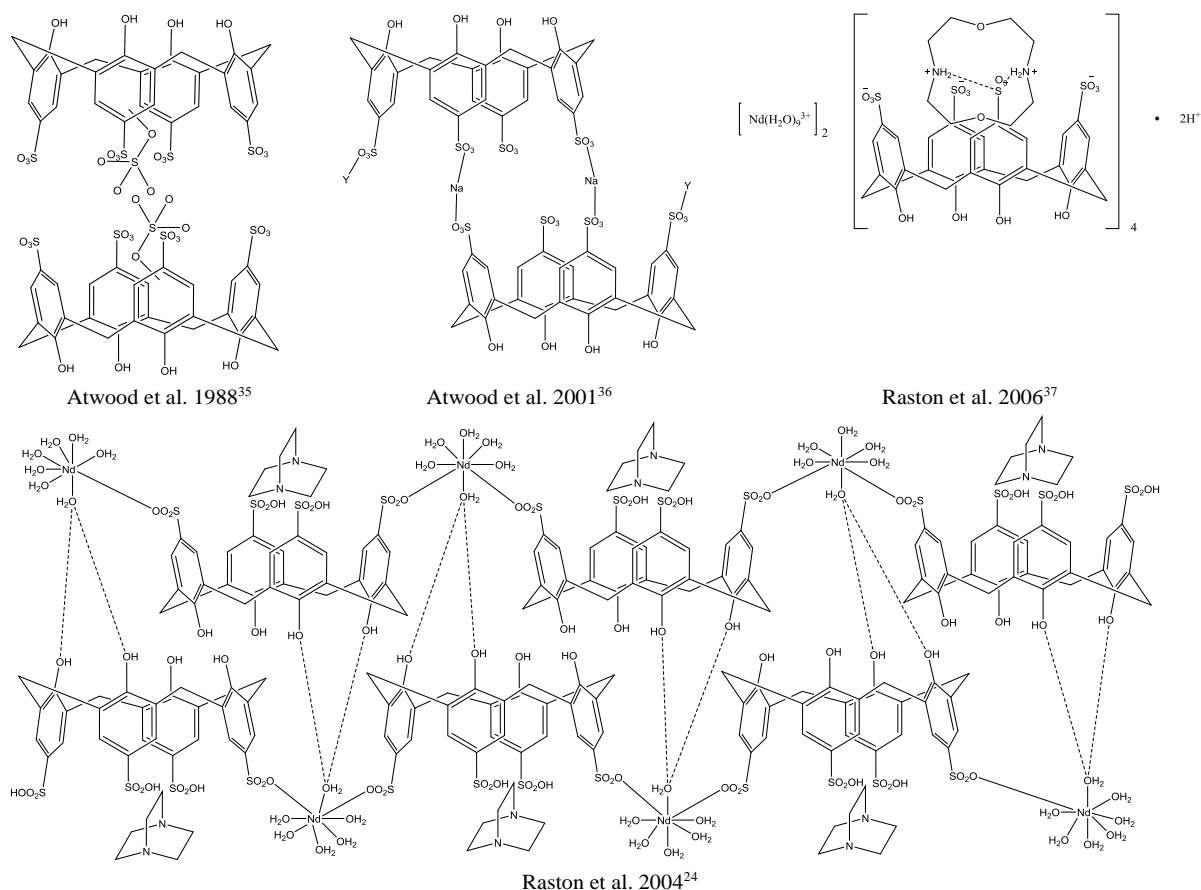


Fig. 3 Structures of similar compounds from the literature

Luminescence studies

Complexes **(1)** (Tb), **(3)** (Eu), **(5)** (Eu), **(6)** (Tb), **(8)** (Eu), and **(10)** (Tb), were analysed in order to determine whether the complexes are suitable for use in optical imaging techniques. Gd complexes are more suited to magnetic imaging techniques and therefore luminescent studies have been omitted. Excitation wavelengths in the range $\lambda_{\text{ex}} = 320\text{--}324$ nm were determined (Fig. S6–S8, ESI) for each complex corresponding to the SC4A.

The emission spectra for **(1)**, **(6)**, and **(10)** showed a mixed spectrum with typical Tb(III) emission maximum at $\lambda_{\text{em}} = 544$ nm overlapping the natural emission from the calixarenes (Fig. S5, ESI), $\lambda_{\text{ex}} = 410$ nm, as shown in Fig. 4, suggesting partial energy transfer from the triplet state of that of the 5D_6 of Tb(III). A similar phenomenon was seen by Stasiuk *et al.* with dual-modal based probes.³⁹ Interestingly for **(3)**, **(5)**, and **(8)** there is no Eu(III) emission observed suggesting the triplet state of the SC4A is too high in energy to efficiently excite the 5D_0 of Eu(III).

By adding a short time delay of 0.05 ms, the fluorescence of the ligand is removed, as the fluorescent lifetime of the organic ligand (μs) is far shorter than that of the lanthanide complex (ms). The time delay reveals characteristic terbium peaks in the spectra (Fig. 5), allowing for dual fluorescent on to different time scales.

By measuring the luminescence decay of these complexes in deuterium oxide and then comparing it to the decay in water it

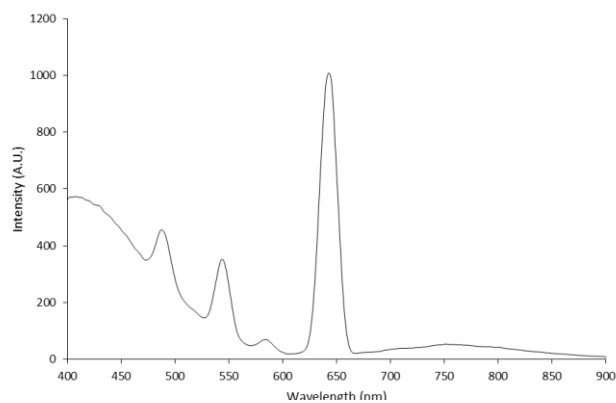


Fig. 4 The emission spectrum of **(1)** without any time delay

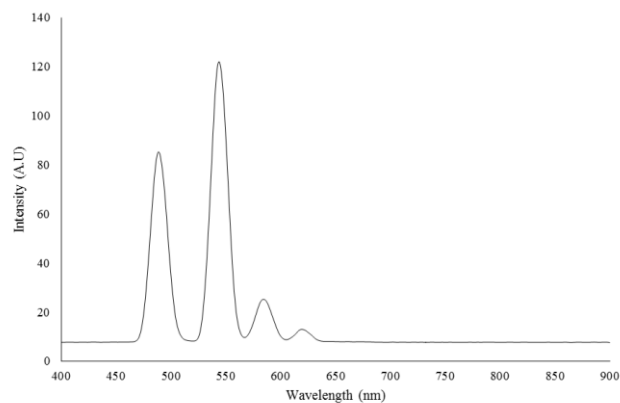


Fig. 5 The emission spectrum of **(1)** with a 0.05 ms delay.

is possible to determine the number of water molecules in the inner coordination sphere of the terbium atoms (q_{Tb}). An example of the decay curves is shown in **Fig. 6** (other decay curves **Fig. S3-S4**, ESI). The number of water molecules in the inner coordination sphere of the terbium atom can be calculated using the following equation:⁴⁰

$$q_{Tb} = 5[(k_{H_2O} - k_{D_2O}) - 0.06]$$

Where k_{H_2O} and k_{D_2O} are determined from the decay curves. In the case of complex **(1)** $k_{H_2O} = 6.457$ and $k_{D_2O} = 5.394$ and therefore q_{Tb} is calculated as 5(.015). The value calculated is an average for the entire sample and suggests that each terbium ion may coordinate up to four calixarene molecules in a monodentate manner. This analysis was also completed for complexes **(6)** and **(10)**, giving q_{Tb} as 9(.146) and 8(.132) respectively. These values indicate monodentate coordination to each calixarene molecule and compliments the crystal structure data.

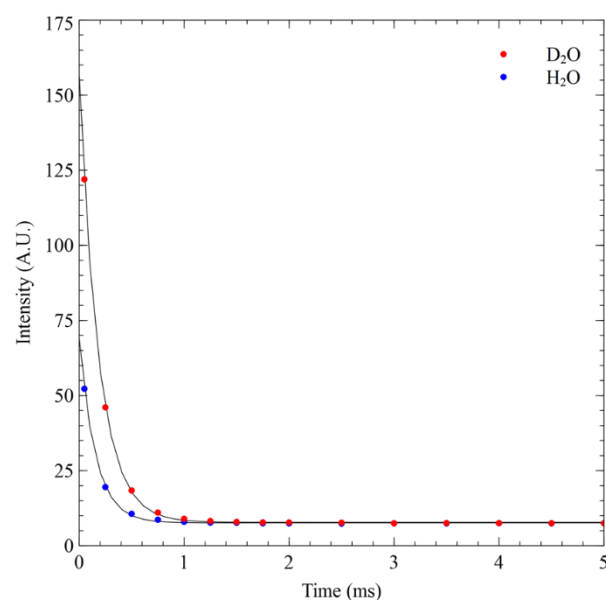


Fig. 6 The luminescence decay curves for **(1)**.

Crystal structure determination

SC4A (Ligand)

A pale brown crystal was extracted from the powdery product after slow evaporation of the solvent. Several small crystals were examined and all those examined were aggregates rather than single crystals. The best of the crystals was chosen for data collection. The crystal examined showed weak scattering and a long exposure time was necessary (12 minutes per 1° ω -rotation frame). The crystal twinning was treated using Rotax. A small

portion of the data suspected to be partially overlapped was omitted from the final refinement.

The structure contains the sulfonated calixarene, water, and dichloromethane (the asymmetric unit is shown in **Fig. S1**, ESI). The calixarene bowls are assembled into dimers by hydrogen bonding to water. The water is located on the outside of these dimers and crystallographically-disordered dichloromethane is found within the cavity formed by the two bowls. Supplementary C-H \cdots π interactions are present between the calixarenes. A portion of the crystal structure is shown in **Fig. 7**.

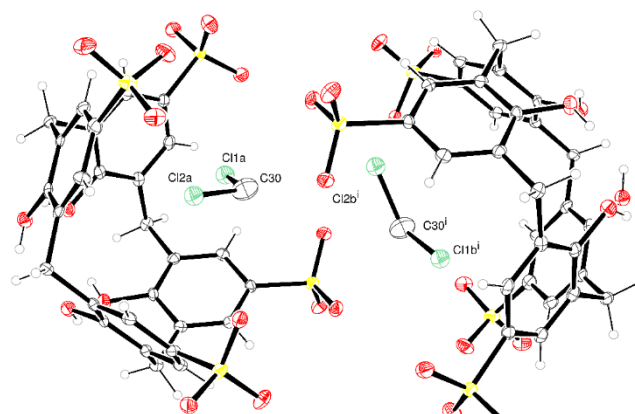


Fig. 7 Centrosymmetric dimer of two calixarenes encapsulating dichloromethane. Atoms are drawn as 30% probability ellipsoids. The right hand calixarene is generated by the symmetry operator $i = 1-x, 1-y, 1-z$. Two different orientations of the disordered dichloromethane are shown. Water molecules outside the dimer have been omitted for clarity.

$[(SC4A)(Tb)(SC4A)(Tb_2)(SC4A)]_n [(SC4A)(Tb)(SC4A)(Tb_2)(SC4A)]_2$ **(1)**

A colourless crystal was selected for data collection. This was found to be a non-merohedral twin. Data were integrated using two twin components and the refinement used all the recorded data within the SHELX HKLF5 formalism. Inclusion of the second component in the refinement (refined twin fraction 0.16) led to substantial improvement in the quality of the fit.

The asymmetric unit contains six symmetry-unique calixarenes and these are bridged by eight independent terbium ions (**Fig. 8**). This produces two structural motifs. One is an infinite chain one calixarene wide running along b . Pairs of calixarenes are linked by two terbium ions and these double units are linked by a single terbium ion (Tb1>Tb3 in **Fig. 8**). Each terbium ion is coordinated by two sulfonate groups and six water molecules. The second structural motif comprises Z-shaped clusters formed from four independent calixarenes; two terbium ions bridge pairs of calixarenes and these dimers are linked by a single terbium ion (Tb4>Tb8 in **Fig. 8**). The coordination about each terbium ion is completed by water such that each terbium ion is surrounded by two independent sulfonates and six water molecules. The Z-shaped clusters fill the space between the infinite chains. There is further water not bound to terbium that resides between the calixarenes. Hydrogen bonding between water, sulfonate, and the phenol groups helps to knit this arrangement together. There is also

evidence for C-H... π interactions between the calixarenes. The infinite chain is illustrated in Fig. 9.

[[Gd(H₂O)₇(SC4A)(Na(H₂O))₂(SC4A)(Gd(H₂O)₇)] (2)

The structure crystallises in the centrosymmetric space group P1 with a single calixarene, one gadolinium ion, one sodium ion,

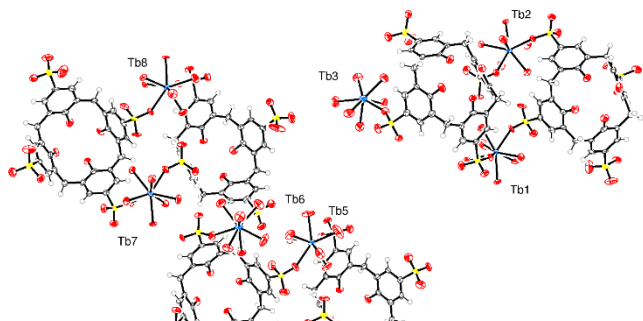


Fig. 8 Asymmetric unit of **(1)**. Atoms drawn as 30% probability ellipsoids. Selected bond lengths: Tb(1)-O(34) 2.388 Å, Tb(1)-O(8) 2.353 Å, Tb(2)-O(11) 2.334 Å, Tb(2)-O(33) 2.396 Å, Tb(3)-O(39) 2.355 Å, Tb(3)-O(1)#1 2.378 Å, Tb(4)-O(102) 2.346 Å, Tb(4)-O(68) 2.378 Å, Tb(5)-O(66) 2.325 Å, Tb(5)-O(93) 2.359 Å, Tb(6)-O(130) 2.371 Å, Tb(6)-O(94) 2.393 Å, Tb(7)-O(162) 2.325 Å, Tb(7)-O(128) 2.353 Å, Tb(8)-O(126) 2.344 Å, Tb(8)-O(151) 2.353 Å.

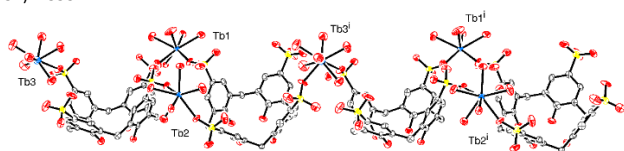


Fig. 9 Portion of the infinite chain within **(1)** that extends along the *b*-axis. Symmetry equivalent atoms are generated by the symmetry operator $i = x, 1+y, z$. (Hydrogen atoms and water are omitted for clarity).

and eleven bound water molecules with further unbound water in the asymmetric unit (Fig. S2, ESI). The gadolinium ion is bound only to one sulfonate and its coordination is completed by seven water molecules to give a square antiprismatic geometry for the gadolinium ion. The water is very well determined (hydrogen atoms located). Pairs of calixarenes are bridged by sodium cations. Each sodium is bound by four water molecules and displays a slightly distorted octahedral geometry with the pair of sulfonate ligands in *cis* configuration at the metal. The water is less well determined and there is some evidence of disorder. The bridging sodium ions produce centrosymmetric dimers of calixarenes as shown in Fig. 10 (the symmetry operation to form the dimer from the asymmetric unit is $i = 1-x, 2-y, -z$). These dimers are packed in a simple primitive sheet arranged in the *yz* plane. There are multiple hydrogen bonds between adjacent dimers (eg two bound waters form hydrogen bonds to sulfonate in the next dimer). The hydrogen bonding is confined within the layers.

These 2D are stacked along *a* with π - π interactions. Specifically, the ring C15>C20 forms π - π interaction with its symmetry equivalent (generated by $i = -x, 2-y, 1-z$); the distance between these means planes is 3.644(3) Å. Secondly the ring, C22>C27 forms π - π interaction with its symmetry equivalent (generated by $i = -x, 2-y, -z$); the distance between these means planes is 3.308(10) Å.

The additional unbound water is located within the cavity formed by the pairs of calixarenes, but none without it. It forms

hydrogen bonds to sulfonate and phenol groups of the calixarenes.

[(SC4A)₂(DO3A)₂Eu(H₂O)₆Na(H₂O)₃.17H₂O] (3) #a

The crystal examined displayed an enormous unit cell, was weakly scattering, displayed atomic disorder and was twinned. Unit cell information: space group I2/a, unit cell parameters $a = 24.4740(8)$ Å, $b = 43.432(2)$ Å, $c = 22.5110(7)$ Å, $\beta = 90.171(3)^\circ$, $V = 23928.3(15)$ Å³.

We have not been able to obtain a crystallographic model in which all the intricate details are resolved.

However it is possible to extract chemically useful data from the refinements. The asymmetric unit has the approximate composition (SC4A)₂(DO3A)₂Eu(H₂O)₆Na(H₂O)₃.17H₂O. The europium ions are bound by pairs of sulfonates from adjacent calixarenes to form dimers. The coordination about the europium ions is completed by water. The DO3A ligand is included in the structure but does not bind to the europium ions, instead it resides within the calixarene cavity. Two carboxylate arms of the DO3A molecule bind the sodium ion (Fig. 11). In a similar fashion to the other structures, a large number of water molecules are present outside the calixarene and these form an extensive hydrogen-bonding network.

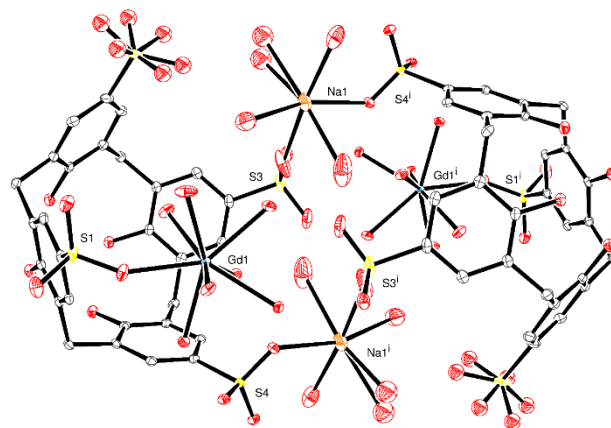


Fig. 10 Centrosymmetric dimer formed from two calixarenes bridged by sodium ions. Symmetry equivalent atoms are generated by the symmetry operator $i = 1-x, 2-y, -z$. Selected bond lengths: Gd(1)-O(5) 2.376 Å, Na(1)-O(11) 2.453 Å, Na(1)-O(14)#1 2.469 Å.

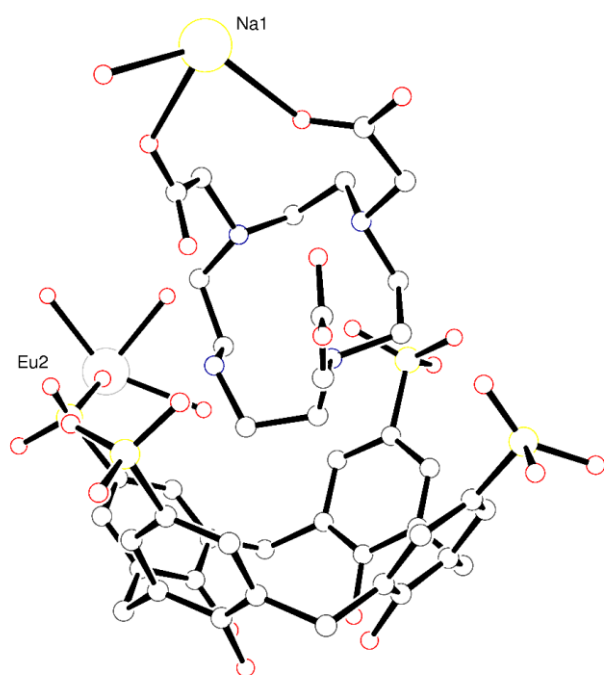


Fig. 11 A portion of the asymmetric unit of **(3)** showing a DO3A molecule within the calixarene bowl, but not binding to europium.

$[\text{Gd}_{13}(\text{SC4A})_7(\text{H}_2\text{O})_{44}] \text{ (4)} \cdot \text{H}_2\text{O}$

Complex **(4)** displays an enormous asymmetric unit with approximate composition $\text{Gd}_{13}(\text{SC4A})_7(\text{H}_2\text{O})_{44}$ and centrosymmetric triclinic unit cell with parameters $a = 15.7674(10) \text{ \AA}$, $b = 25.4062(12)$, $c = 49.535(3) \text{ \AA}$, $\alpha = 103.035(4)^\circ$, $\beta = 94.600(5)^\circ$, $\gamma = 107.212(4)^\circ$, $V = 18234.4(19) \text{ \AA}^3$. Within the asymmetric unit there are 13 independent Gd ions. The huge unit cell and presence of many unbound water molecules make this very challenging. It is possible to extract chemical information from this data set, although refinements are poor. The structure contains the expected calixarenes and the sulfonate groups coordinate to the gadolinium ions. The mode of bridging is similar to the other structures and the gadolinium ions link together the calixarenes to form dimers and trimers rather than polymers. The coordination about the gadolinium ions is completed by water. See Fig. 12 for an illustration of a portion of the structure.

Cytotoxicity studies

In order to use the optical imaging agents for future *in vivo* applications, it is essential to have an indication of toxicity. Given the potential biomedical applications of SCnAs, toxicity studies have been reported by Paquet *et al.* In particular they studied the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in polymorphonuclear neutrophils (PMNs), and observed that the compounds did not stimulate the neutrophils.²⁷ Subsequent *in vivo* experiments were carried out on 35S radiolabelled SC4A, showing no toxicity at 100 mg

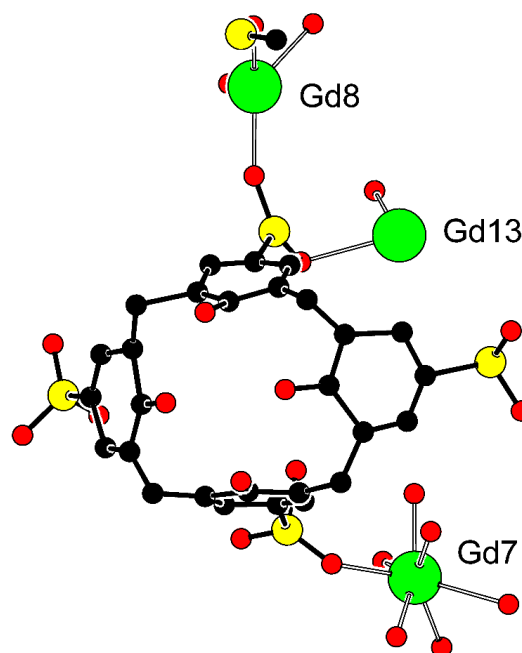


Fig. 12 A portion of the asymmetric unit of **(4)** demonstrating the presence of sulfonated calixarenes that are bound to gadolinium ions.

kg^{-1} , equivalent to a dose of 2-5 g for humans.²⁸ To ensure the lanthanide complexation in this study did not have a toxicity level that would negatively impact on *in vitro* cellular studies, we have performed a cell viability assay. Complexes **(1) - (10)** were tested for their anti-proliferation activity against OE33 cells. These cells were incubated for 70 h before determining their mitochondrial based reduction of a tetrazolium dye (MTS) to a formazan product which absorbs at 490 nm. This experiment was carried out over a range of concentrations to determine the amount of compound required to reduce cell growth by 50% (cytotoxic concentration, CC_{50}). All compounds in this study are relatively non-toxic with CC_{50} values in the range 30-170 μmol . Dose response curves for treatment of OE33 cells with compounds **(1) - (10)** are shown in Fig. 13. These data are encouraging toward future biomedical applications. However, there is a preference for coordination of the lanthanide in the tetraazamacrocycle cavity for *in vivo* studies due to increased stability. This will be the subject of future studies.

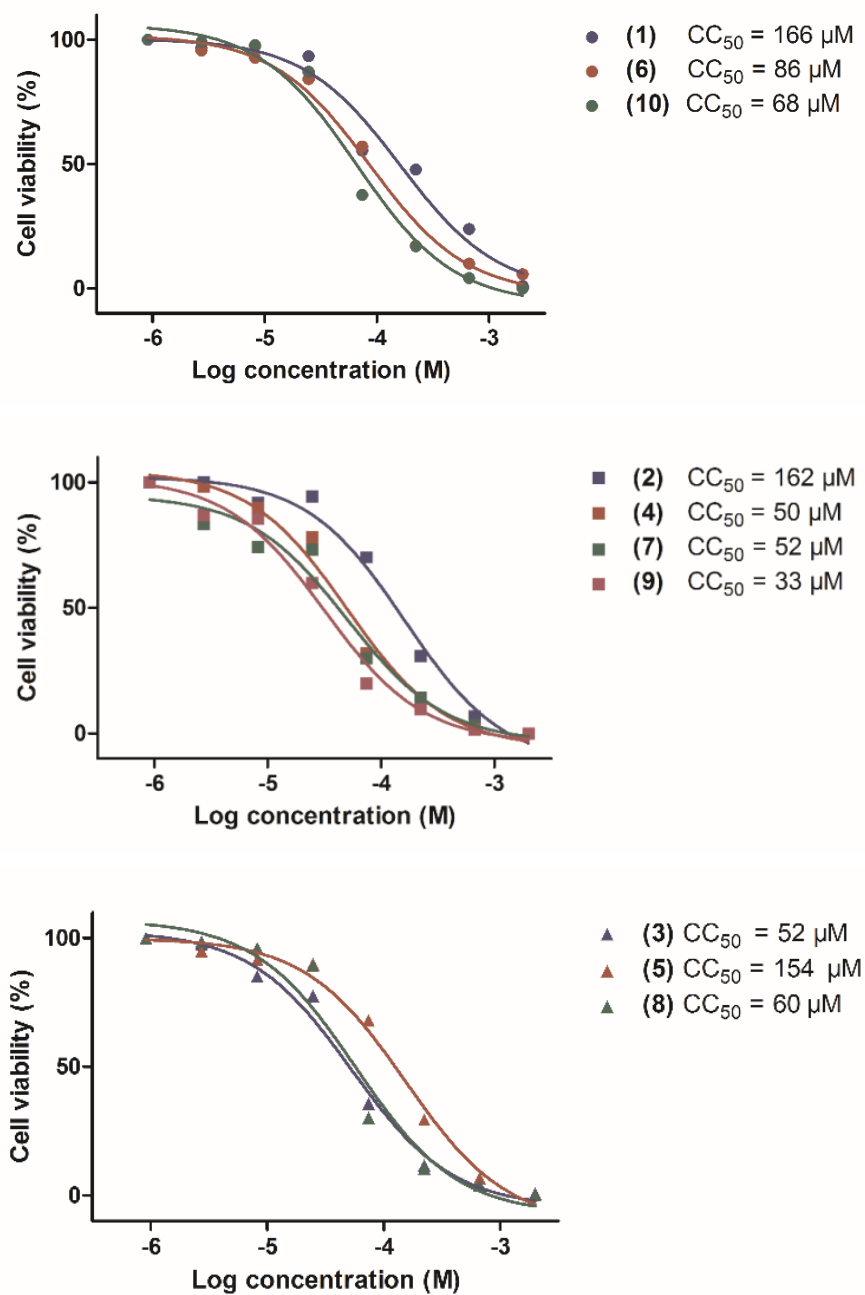


Fig. 13 MTS assay results (top = terbium complexes, middle = gadolinium complexes, bottom = europium complexes).

Compound	SC4A	(1)	(2)
Formula	C ₂₉ H ₃₂ Cl ₂ O ₂₃ S ₄	C ₁₆₈ H ₉₆ O ₁₉₅ S ₂₄ Tb ₈	C ₅₆ H ₆₈ Gd ₂ Na ₂ O ₆₇ S ₈
Formula weight	947.68	7275.24	2430.06
Crystal system	Triclinic	Triclinic	Triclinic
Space group	P -1	P -1	P -1
Unit cell dimensions			
<i>a</i> (Å)	11.9482(10)	16.504(4)	12.2240(3)
<i>b</i> (Å)	12.3059(8)	23.227(3)	13.6903(3)
<i>c</i> (Å)	15.2457(12)	38.406(7)	14.0756(9)
α (°)	68.694(5)	73.798(12)	90.710(6)
β (°)	77.073(6)	80.363(16)	105.688(7)
γ (°)	89.160(6)	85.658(15)	90.843(6)
<i>V</i> (Å ³)	2030.0(3)	13932(.5)	2267.23(18)
<i>Z</i>	2	2	1
Temperature (K)	150(2)	150(2)	100(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Calculated density (Mg m ⁻³)	1.550	1.724	1.780
Absorption coefficient (mm ⁻¹)	0.452	2.301	1.762
Transmission factors (min/max)	0.925 and 0.982	0.483 and 0.768	0.722 and 1.000
Crystal size (mm ³)	0.240 x 0.060 x 0.055	0.330 x 0.310 x 0.100	0.120 x 0.060 x 0.020
ϑ (max) (°)	25.433	25.316	27.483
Reflections measured	11167	156086	30671
Unique reflections	11167	156086	10385
<i>R</i> _{int}	0.068	0.0571	0.0294
Reflections with <i>F</i> ² > 2σ(<i>F</i> ²)	5027	69332	10004
Number of parameters	568	3557	645
<i>R</i> ₁ [<i>F</i> ² > 2σ(<i>F</i> ²)]	0.0676	0.0838	0.0443
<i>wR</i> ₂ (all data)	0.1760	0.2453	0.1110
GOOF, <i>S</i>	0.828	0.823	1.120
Largest difference peak and hole (e Å ⁻³)	1.248 and -1.217	8.675 and -3.446	3.662 and -1.191

Table 2 Crystal data for the ligand, complex (1), and complex (2)

Conclusions

A series of lanthanide (Tb, Gd, and Eu) based SC4A complexes have been synthesised, characterised and screened for their anti-proliferation activity. Luminescence studies shows that the SC4A triplet state is too high in energy to efficiently excite the Eu based complexes, however has the ability to excite the Tb based complexes. The biological screening shows low CC_{50} values for all complexes tested suggesting that with further development complexes of this type could be used for biomedical applications.

Experimental section

General remarks

NMR spectra were recorded on a Jeol JNM ECP400 spectrometer and a Jeol JNM-LA400, with TMS $\delta_H = 0$ ppm as the internal standard or residual protic solvent [D_2O , $\delta_H = 4.79$; $CDCl_3$, $\delta_H = 7.26$]. Chemical shifts are given in ppm (δ) and coupling constants (J) are given in Hertz (Hz). Peak types in the spectra are denoted by the following notations: broad (br), singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sex), and multiplet (m).

Elemental analysis were carried out by staff at the University of Hull.

Infrared spectra were recorded on a Nicolet iS5 FT-IR spectrometer.

Emission and excitation spectra were recorded on a Perkin Elmer LS 55 fluorescence spectrometer.

X-Ray diffraction data were collected on a STOE IPSD2 image plate diffractometer (Hull) or a Rigaku diffractometer (Swansea).

Mass spectra were obtained from the EPSRC National Mass Spectrometry Service Unit, Swansea University.

p-Sulfonatocalix[4]arene and 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid were prepared using previously reported methods.^{24,13}

Lanthanide salts were obtained from Sigma-Aldrich Company Ltd. and used as received.

Synthesis of $[(SC4A)(Tb)(SC4A)(Tb_2)(SC4A)]_n[(SC4A)(Tb)(SC4A)(Tb_2)(SC4A)]_2$ (1)

p-Sulfonatocalix[4]arene (0.5 g, 0.67 mmol) was dissolved in water (10 mL). To this solution was added terbium acetate (0.5 g, 1.49 mmol) in water (15 mL). The resulting solution was heated at 95°C for 2 h and then left to stir at room temperature overnight. The solution was concentrated *in vacuo* to give a buff powder (78 mg, 10.7%). Found: C, 30.08; H, 3.47; S, 11.98. Calcd. for $C_{196}H_{218}Tb_8O_{144}.40(H_2O)$: C, 30.31; H, 3.87; S, 11.56%. ν_{max}/cm^{-1} 3222 (w, br) H_2O , 1152 (s) SO_3^- , 1121 (s), 1038 (s) SO_3H . m/z 1035.3399 (SC4A-Tb(OH)₈-SC4A-Tb(OH)₈). ¹H NMR (400 MHz, D_2O) $\delta = 5.60$ (br s, 8 H, Ar-*H*), 2.86 (br s, 8 H, Ar-*CH_2*-Ar).

Synthesis of $[(Gd(H_2O)_7)(SC4A)(Na(H_2O))_2(SC4A)(Gd(H_2O)_7)]$ (2)

Gd-DO3A (0.20 g, 0.4 mmol) was dissolved in water (5 mL). To this solution was added *p*-sulfonatocalix[4]arene (0.30 g, 0.4 mmol). The resulting mixture was heated to 95°C for 4 h. After cooling, the solution was neutralised with aqueous 5% sodium hydroxide solution following which a precipitate forms, which was removed by filtration. The solution was concentrated *in vacuo* to give an off-white powder (44 mg, 10.7%). Found: C, 31.31; H, 3.54; S, 11.28. Calcd. for $3[C_{56}H_{72}Gd_2NaO_{46}S_8][C_{14}H_{22}GdNa_4O_{16}].16(H_2O)$: C, 31.36; H, 3.30; S, 11.04%. ν_{max}/cm^{-1} 3225 (w, br) H_2O , 1150 (s) SO_3^- , 1122 (s), 1041 (s) SO_3H . m/z 457.4448 (SC4A-Gd(OH)).

Synthesis of $[(SC4A)_2(DO3A)_2Eu(H_2O)_6Na(H_2O)_3.17(H_2O)]$ (3)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by europium acetate (0.22 g, 0.67 mmol). The solution was then stirred at 90°C for 4 h. After cooling, the solution was neutralised with aqueous 5% sodium hydroxide solution and solid by-products were removed by filtration. The solution was concentrated *in vacuo* to give a cream powder (71 mg, 8.1%). Found: C, 35.02; H, 4.81; N, 3.92; S, 8.78. Calcd. for $C_{42}H_{47}EuNaO_{22}S_4.10(H_2O)$: C, 34.95; H, 4.68; N, 3.88; S, 8.89%. ν_{max}/cm^{-1} 3215 (w, br) H_2O , 1151 (s) SO_3^- , 1122 (s), 1041 (s) SO_3H . m/z 619.0297 (SC4A-Eu-DO3A). ¹H NMR (400 MHz, D_2O) $\delta = 7.46$ (s, 8 H, Ar-*H*), 3.91 (s, 8 H, Ar-*CH_2*-Ar), 3.73, 2.94 (t, 8 H, N-*CH_2*-*CH_2*-N), 2.38 (s, 6 H, N-*CH_2*-COOH), 2.28 (m, 2 H, N-*CH_2*-*CH_2*-N), 2.04 (m, 2 H, N-*CH_2*-*CH_2*-N), 1.90 (m, 2 H, N-*CH_2*-*CH_2*-N), 1.59 (m, 2 H, N-*CH_2*-*CH_2*-N).

Synthesis of $[Gd_{13}(SC4A)_7(H_2O)_{44}]$ (4)

p-Sulfonatocalix[4]arene (1.0 g, 1.34 mmol) was dissolved in water (20 mL). To this solution was added gadolinium acetate (1.0 g, 2.99 mmol). The resulting solution was heated at 95°C for 4 h. After 30 min a precipitate had formed, which was removed upon completion of the heating period. The filtrate was concentrated *in vacuo* to give a cream powder (100 mg, 7.7%). Found: C, 29.55; H, 3.55; S, 11.98. Calcd. for $C_{224}H_{194}Gd_4O_{132}S_{33}.48(H_2O)$: C, 29.49; H, 3.20; S, 11.60%. ν_{max}/cm^{-1} 3265 (w, br) H_2O , 1151 (s) SO_3^- , 1119 (s), 1035 (s) SO_3H . m/z 1035.3399 (SC4A-(Gd(OH)₈)₂-SC4A).

Synthesis of $[(Eu(H_2O)_9)(SC4A)(Eu(H_2O)_8)(SC4A)(Eu(H_2O)_9)]$ (5)

p-Sulfonatocalix[4]arene (0.5 g, 0.67 mmol) was dissolved in water (10 mL). To this solution was added europium acetate (0.5 g, 1.52 mmol) in water (15 mL). The resulting solution was heated at 95°C for 2 h and then left to stir at room temperature overnight. The solution was concentrated *in vacuo* to give a cream powder (107 mg, 19.8%). Found: C, 28.29; H, 3.97; S, 10.59. Calcd. for $C_{57}H_{97}O_{57}S_8Eu_3.50(H_2O)$: C, 28.45; H, 4.06; S,

10.66%, $\nu_{\max}/\text{cm}^{-1}$ 3248 (w, br) H₂O, 1155 (s) SO₃⁻, 1119 (s), 1040 (s) SO₃H. m/z 1035.3403 (SC4A-Eu(OH)₃(H₂O)₅). ¹H NMR (400 MHz, D₂O) δ 7.52 (s, 8 H, Ar-H), 3.78 (s, 8 H, Ar-CH₂-Ar).

Synthesis of

[(Tb(H₂O)₉)(SC4A)(Tb(H₂O)₈)(SC4A)(Tb(H₂O)₉)(DO3A).22(H₂O)] (6)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by terbium acetate (0.23 g, 0.67 mmol). The solution was then stirred at 90°C for 4 h. Upon cooling a precipitate formed, which was removed by filtration. The filtrate was concentrated *in vacuo* to give a cream powder (110 mg, 17.7%). Found: C, 30.15; H, 4.40; N, 1.95; S, 9.37. Calcd. for C₇₀H₁₂₂N₄O₆₄S₈Tb₃.22(H₂O): C, 30.28; H, 4.43; N, 2.02; S, 9.24%, $\nu_{\max}/\text{cm}^{-1}$ 3211 (w, br) H₂O, 1151 (s) SO₃⁻, 1116 (s), 1038 (s) SO₃H. m/z 414.3518 (Tb(OH)₆-SC4A-Tb(OH)₅).

Synthesis of

[(Gd(H₂O)₉)(SC4A)(Gd(H₂O)₈)(SC4A)(Gd(H₂O)₉)(DO3A).19 (H₂O)] (7)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by gadolinium acetate (0.23 g, 0.67 mmol). The solution was then stirred at 90°C for 4 h. Upon cooling a precipitate formed, which was removed by filtration. The filtrate was concentrated *in vacuo* to give a cream powder (43 mg, 6.9%). Found: C, 30.19; H, 4.52; N, 2.06; S, 9.40. Calcd. for C₇₀H₁₂₂N₄O₆₄S₈Gd₃.19(H₂O): C, 30.33; H, 4.44; N, 2.02; S, 9.25%, $\nu_{\max}/\text{cm}^{-1}$ 3217 (w, br) H₂O, 1148 (s) SO₃⁻, 1116 (s), 1037 (s) SO₃H. m/z 1035.3402 (SC4A-Gd(OH)₈).

Synthesis of [(SC4A)(Eu(H₂O)₈)(SC4A)(Eu₂(H₂O)₁₆)(SC4A).35(H₂O)]_n (8)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by europium acetate (0.44 g, 1.34 mmol). The solution was then stirred at 90°C for 4 h. Upon cooling a precipitate formed, which was removed by filtration. The filtrate was concentrated *in vacuo* to give a cream powder (182 mg, 26.1%). Found: C, 32.25; H, 3.74; S, 12.20. Calcd. for C₈₄H₁₁₄O₇₂S₁₂Eu₃.35(H₂O): C, 32.37; H, 3.69; S, 12.35%, $\nu_{\max}/\text{cm}^{-1}$ 3232 (w, br) H₂O, 1150 (s) SO₃⁻, 1117 (s), 1038 (s) SO₃H. m/z 1035.3408 (SC4A-Eu(OH)₃(H₂O)₅). ¹H NMR (400 MHz, D₂O) δ 7.39 (s, 8 H, Ar-H), 3.97 (s, 8 H, Ar-CH₂-Ar)

Synthesis of [(SC4A)(Gd(H₂O)₈)(SC4A)(Gd₂(H₂O)₁₆)(SC4A).44(H₂O)]_n (9)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by gadolinium acetate (0.46 g, 1.34 mmol). The solution was then

stirred at 90°C for 4 h. Upon cooling a precipitate formed, which was removed by filtration. The filtrate was concentrated *in vacuo* to give a cream powder (274 mg, 39.2%). Found: C, 30.01; H, 5.23; S, 10.03. Calcd. for C₈₄H₁₁₄O₇₂S₁₂Gd₃.44(H₂O): C, 25.94; H, 5.13; S, 9.89% $\nu_{\max}/\text{cm}^{-1}$ 3201 (w, br) H₂O, 1149 (s) SO₃⁻, 1116 (s), 1037 (s) SO₃H. m/z 1035.3416 (SC4A-Gd(OH)₈).

Synthesis of [(SC4A)(Tb(H₂O)₈)(SC4A)(Tb₂(H₂O)₁₆)(SC4A).31(H₂O)]_n (10)

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added *p*-sulfonatocalix[4]arene (0.5 g, 0.67 mmol) followed by terbium acetate (0.44 g, 1.34 mmol). The solution was then stirred at 90°C for 4 h. Upon cooling a precipitate formed, which was removed by filtration. The filtrate was concentrated *in vacuo* to give a cream powder (95 mg, 13.6%). Found: C, 31.99; H, 3.54; S, 12.31. Calcd. for C₈₄H₁₁₄O₇₂S₁₂Tb₃.31(H₂O): C, 32.16; H, 3.66; S, 12.26% $\nu_{\max}/\text{cm}^{-1}$ 3201 (w, br) H₂O, 1149 (s) SO₃⁻, 1117 (s), 1038 (s) SO₃H. m/z 1035.3404 (SC4A-Tb(H₂O)₈-SC4A-Tb(H₂O)₉).

Crystal structure determinations

Crystals suitable for X-ray diffraction were grown by complete dissolution of the complexes **(1) – (4)** in water and allowing the water to slowly evaporate. For the ligand (SC4A), the solid was briefly heated in dichloromethane and filtered whilst hot. The solvent was then allowed to slowly evaporate leaving pale brown crystals amongst a pale brown powder.

For SC4A, **(1)**, **(3)**, and **(4)**, single crystal X-ray diffraction data were collected in a series of ω scans using a STOE IPSD2 image plate diffractometer utilising monochromated molybdenum radiation ($\lambda = 0.71073 \text{ \AA}$). Standard procedures were employed for the integration and processing of the data using X-RED.²⁹ Samples were coated in a thin film of perfluoropolyether oil and mounted at the tip of a glass fibre located on a goniometer. Data were collected from crystals held at 150K in an Oxford Instruments nitrogen gas cryostream.

Crystal structures were solved using routine automatic direct methods implemented within SHELXS-97.³⁰ Completion of structures was achieved by performing least squares refinement against all unique F² values using SHELX-97.³⁰

For **(2)**, data were collected by the EPSRC National Crystallography Service³¹ using a Rigaku diffractometer with a molybdenum rotating anode and Rigaku Saturn724+ detector. The crystal was kept at 100K for the data collection.

Cytotoxicity

MTS assay is based upon the conversion of tetrazolium salt to into formazan in viable cells via mitochondrial dehydrogenase enzyme activity. The amount of formazan is directly proportional to the number of viable cells in the culture media. OE33 (oesophageal carcinoma) cells were seeded in 96 flat bottomed microtiter tissue culture plates with 1000 cells/well in 200 μL media (RPMI + 1% glutamine + 10% FCS). The plates

were incubated overnight in a 5% CO₂ incubator at 37°C to allow cells to adhere. The next day the media was removed from the wells and 100 µL compound in media was added. Tested compounds were used in a range of concentrations from 0.91 µM to 2 mM. The plates were then returned to a 5% CO₂ incubator for 72 h after which MTS reagent (Promega, UK) 20 µL was added to each well and returned to the incubator at 37°C for a further 3 h. Absorbance readings were taken at 490 nm using a Synergy HT microplate reader (Biotek, USA). Experiments were carried out in triplicate and subtracted from media only absorbance. CC₅₀ values were obtained using GraphPad Prism 5 (GraphPad, USA) software.

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We would like to thank the EPSRC Mass Spectrometry Service Centre at Swansea University for the collection of mass spectra.

Notes and references

‡ CCDC 1449711 - 1449713 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

‡^a Complex (**3**) displayed an enormous unit cell and was twinned. It displayed atomic disorder and there are large regions of the structure contain diffuse electron density, probably due to poorly-ordered water molecules. We do not claim this structure is finished and suitable for deposition in the CCDC or wish to report the refinement details. However, refinements against the data available give (we believe unequivocally) useful chemical information about the nature of the molecules present and their coordination to europium and sodium ions.

‡^b Complex (**4**) has similar problems: a huge unit cell including 13 crystallographically independent gadolinium ions and large volumes of space occupied by poorly-ordered solvent. As for (**3**), we do not consider that we have derived a model of the complete crystal structure but we can identify key chemically-intelligible features that add to our understanding of this compound. We do not claim this structure is finished and suitable for deposition in the CCDC or wish to report the refinement details.

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