
David M. Miller-Shakesby$^a$, Benjamin P. Burke$^a$, Shubhanchi Nigam$^a$, Graeme J. Stasiuk$^b$, Timothy J. Prior$^a$, Stephen J. Archibald$^{a,b}$,* and Carl Redshaw$^{a,*}$

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 CC$_{50}$ values in the range $30 – 170 \, \mu$M.

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 the first reported water soluble calixarene was reported by Ungaro et al.\textsuperscript{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, SC$n$As) are a class of highly water-soluble calixarene first reported by Shinkai et al.\textsuperscript{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.\textsuperscript{3} Previous studies have shown that SC$n$As are biocompatible\textsuperscript{4,5}, in that they are both non-toxic and water-soluble, which makes them eligible for biological and pharmaceutical applications.\textsuperscript{6,7} For these reasons, we have selected SC$n$As for the present study.

A considerable amount of work on SC4A lanthanide complexes has been carried out previously.\textsuperscript{8-14} Work by Atwood et al.\textsuperscript{8,15} 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.\textsuperscript{15} These assemblies consist of crown ether molecules sat within the hydrophobic cavity created by two calixarene molecules.\textsuperscript{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 SC$n$A molecules.\textsuperscript{16-22}

Further work by Dalgarno and Atwood, describes a coordination polymer formed with alternating dipyridine/SC6As linked with lanthanide cations, which also consists of a bi-layer arrangement.\textsuperscript{23} It was also demonstrated that by controlling the pH, structures where two SC4As envelop a crown ether molecule can be obtained.\textsuperscript{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.\textsuperscript{25}

Another macrocycle often used for biological applications is 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A, SC4A showing the exo and endo rims, and DO3A.
shown Fig. 1.26 Synthesised from the reaction between 1,4,7,10-tetraazacyclododecane (cyclen) and t-butylibromocacetate,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 T1 relaxation 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 R1 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 tetraaza macrocycle 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-tetraaza cyclododecane (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 tetraaza macrocycles 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.35,36 Complexes (1), (4), and (5) were prepared by heating an aqueous solution of Ln(O2C2H5)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.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Ln salt (eq.)</th>
<th>SC4A (eq.)</th>
<th>DO3A (eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>2.2 (Tb)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(2)</td>
<td>1 (Gd)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(3)</td>
<td>1 (Eu)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(4)</td>
<td>2.2 (Gd)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(5)</td>
<td>2.2 (Eu)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(6)</td>
<td>1 (Tb)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(7)</td>
<td>1 (Gd)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(8)</td>
<td>2 (Eu)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(9)</td>
<td>2 (Gd)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(10)</td>
<td>2 (Tb)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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.
Comparison of the infrared spectrum of the SC4A ligand with those of the lanthanide complexes reveals a reduction in the intensity of the SO\textsubscript{3}H band (~1035 cm\textsuperscript{-1}), whereas all other bands remain unaffected. This also confirms coordination of the lanthanide ion to the SO\textsubscript{3}H group on the exo rim of the SC4A.
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 $\lambda_{\text{ex}} = 320 - 324$ 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), $\lambda_{\text{em}} = 410$ 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. 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...
is possible to determine the number of water molecules in the inner coordination sphere of the terbium atoms ($q_{\text{Tb}}$). An example of the decay curves is shown in Fig. 6 (other decay curves Fig. 5-54, ESI). The number of water molecules in the inner coordination sphere of the terbium atom can be calculated using the following equation:

$$q_{\text{Tb}} = 5\left(k_{\text{H}_2\text{O}} - k_{\text{D}_2\text{O}} - 0.06\right)$$

Where $k_{\text{H}_2\text{O}}$ and $k_{\text{D}_2\text{O}}$ are determined from the decay curves. In the case of complex (1) $k_{\text{H}_2\text{O}} = 6.457$ and $k_{\text{D}_2\text{O}} = 5.394$ and therefore $q_{\text{Tb}}$ 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 $q_{\text{Tb}}$ as 9(.146) and 8(.132) respectively. These values indicate monodentate coordination to each calixarene molecule and compliments the crystal structure data.

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···π interactions are present between the calixarenes. A portion of the crystal structure is shown in Fig. 7.

![Fig. 7](image.png)

**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.

$$\text{[(SC4A)(Tb)(SC4A)(Tb)(SC4A)](1)}$$

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.

\[ ([\text{Gd(H}_2\text{O})_6\text{SC4A}]\text{Na(H}_2\text{O})_2)(\text{SC4A})\text{Gd(H}_2\text{O})_6]) \] (2)

The structure crystallises in the centrosymmetric space group P1 with a single calixarene, one gadolinium ion, one sodium ion, 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 and water are omitted for clarity).

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(14) 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).

Two carboxylate arms of the DO3A molecule bind the sodium ions. 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 (e.g., 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 \( \pi-\pi \) interactions. Specifically, the ring C15>C20 forms \( \pi-\pi \) 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 \( \pi-\pi \) 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.

\[ ([\text{SC4A}]_2\text{DO3A})_2\text{Eu(H}_2\text{O})_6\text{Na(H}_2\text{O})_2\cdot17\text{H}_2\text{O}) \] (3)

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, \( \beta = 90.171(3)^\circ \). 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 \( (\text{SC4A})_2\text{DO3A})_2\text{Eu(H}_2\text{O})_6\text{Na(H}_2\text{O})_2\cdot17\text{H}_2\text{O} \). 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. 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) 2.469 Å.
Complex (4) displays an enormous asymmetric unit with approximate composition Gd_{13}(SC4A)_{7}(H_{2}O)_{44} and centrosymmetric triclinic unit cell with parameters a = 15.7674(10) Å, b = 25.4062(12), c = 49.535(3) Å, α = 103.035(4)°, β = 94.600(5)°, γ = 107.212(4)°, V = 18234.4(19) Å$^3$. 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.

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 Paclet 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. Subsequent in vivo experiments were carried out on 35S radiolabelled SC4A, showing no toxicity at 100 mg kg$^{-1}$, equivalent to a dose of 2-5 g for humans. 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).
<table>
<thead>
<tr>
<th>Compound</th>
<th>SC4A</th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>$\text{C}<em>{29}\text{H}</em>{32}\text{Cl}<em>{2}\text{O}</em>{2}\text{S}_{4}$</td>
<td>$\text{C}<em>{168}\text{H}</em>{96}\text{O}<em>{48}\text{S}</em>{24}\text{TB}_{8}$</td>
<td>$\text{C}<em>{56}\text{H}</em>{48}\text{Gd}<em>{2}\text{Na}</em>{2}\text{O}<em>{87}\text{S}</em>{8}$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>947.68</td>
<td>7275.24</td>
<td>2430.06</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
<td>Triclinic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P -1</td>
<td>P -1</td>
<td>P -1</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>11.9482(10)</td>
<td>16.504(4)</td>
<td>12.2240(3)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>12.3059(8)</td>
<td>23.2273(3)</td>
<td>13.6903(3)</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>15.2457(12)</td>
<td>38.4067(7)</td>
<td>14.0756(9)</td>
</tr>
<tr>
<td>$\alpha$ (Å)</td>
<td>68.694(5)</td>
<td>73.79812(12)</td>
<td>90.7106(6)</td>
</tr>
<tr>
<td>$\beta$ (Å)</td>
<td>77.073(6)</td>
<td>80.36316(12)</td>
<td>105.6887(6)</td>
</tr>
<tr>
<td>$\gamma$ (Å)</td>
<td>89.160(6)</td>
<td>85.65815(12)</td>
<td>90.8436(6)</td>
</tr>
<tr>
<td>$V$ (Å$^3$)</td>
<td>2030.00(3)</td>
<td>13932.50(1)</td>
<td>2267.23(18)</td>
</tr>
<tr>
<td>$Z$</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>150(2)</td>
<td>150(2)</td>
<td>100(2)</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.71073</td>
<td>0.71073</td>
<td>0.71073</td>
</tr>
<tr>
<td>Calculated density (Mg m$^{-3}$)</td>
<td>1.550</td>
<td>1.724</td>
<td>1.780</td>
</tr>
<tr>
<td>Absorption coefficient (mm$^{-1}$)</td>
<td>0.452</td>
<td>2.301</td>
<td>1.762</td>
</tr>
<tr>
<td>Transmission factors (min/max)</td>
<td>0.925 and 0.982</td>
<td>0.483 and 0.768</td>
<td>0.722 and 1.000</td>
</tr>
<tr>
<td>Crystal size (mm$^3$)</td>
<td>0.240 x 0.060 x 0.055</td>
<td>0.330 x 0.310 x 0.100</td>
<td>0.120 x 0.060 x 0.020</td>
</tr>
<tr>
<td>$\vartheta$ (max) ($^\circ$)</td>
<td>25.433</td>
<td>25.316</td>
<td>27.483</td>
</tr>
<tr>
<td>Reflections measured</td>
<td>11167</td>
<td>156086</td>
<td>30671</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>11167</td>
<td>156086</td>
<td>10385</td>
</tr>
<tr>
<td>$R_{int}$</td>
<td>0.068</td>
<td>0.0571</td>
<td>0.0294</td>
</tr>
<tr>
<td>Reflections with $F^2 &gt; 2\sigma(F^2)$</td>
<td>5027</td>
<td>69332</td>
<td>10004</td>
</tr>
<tr>
<td>$\vartheta$ (max) ($^\circ$)</td>
<td>25.433</td>
<td>25.316</td>
<td>27.483</td>
</tr>
<tr>
<td>Number of parameters</td>
<td>568</td>
<td>3557</td>
<td>645</td>
</tr>
<tr>
<td>$R_1 [F^2 &gt; 2\sigma(F^2)]$</td>
<td>0.0676</td>
<td>0.0838</td>
<td>0.0443</td>
</tr>
<tr>
<td>$wR_2$ (all data)</td>
<td>0.1760</td>
<td>0.2453</td>
<td>0.1110</td>
</tr>
<tr>
<td>GOOF, $S$</td>
<td>0.828</td>
<td>0.823</td>
<td>1.120</td>
</tr>
<tr>
<td>Largest difference peak and hole (e Å$^{-3}$)</td>
<td>1.248 and -1.217</td>
<td>8.675 and -3.446</td>
<td>3.662 and -1.191</td>
</tr>
</tbody>
</table>

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 show 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 CC50 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 δH = 0 ppm as the internal standard or residual protic solvent [D2O, δH = 4.79; CDCl3, δH = 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 iSS FT-IR spectrometer. Emission and excitation spectra were recorded on a Perkin Elmer LS 55 fluorescence spectrometer.

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

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 cream powder (71 mg, 8%). Found: C, 35.02; H, 4.81; N: 3.92; S: 8.78. Calcd. for C34H34EuNaO22S6: C, 34.95; H, 4.68; N, 3.88; S, 8.89%. δH NMR (400 MHz, D2O) δ = 7.46 (s, 8 H, Ar-H), 3.91 (s, 8 H, Ar-CH2-Ar), 3.73, 2.94 (t, 2 H, N-CH2-CH2-N), 2.38 (s, 6 H, N-CH2-COOH), 2.28 (m, 2 H, N-CH2-CH2-N), 2.04 (m, 2 H, N-CH2-CH2-N), 1.90 (m, 2 H, N-CH2-CH2-N), 1.59 (m, 2 H, N-CH2-CH2-N).


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 C19H13GdO12S13·48H2O: C, 29.49; H, 3.20; S, 11.60%. δH NMR (400 MHz, D2O) δ = 5.60 (br s, 8 H, Ar-H), 2.86 (br s, 8 H, Ar-CH2-Ar).

Synthesis of [(SC4A)(Na(H2O)4)]2 [2]
Gd-D03A (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[CuH2Gd2Na3O6S8][Cu4H2Gd3N2O16.16H2O]: C, 31.36; H, 3.30; S, 11.04%. δH NMR (400 MHz, D2O) δ = 5.60 (br s, 8 H, Ar-H), 2.86 (br s, 8 H, Ar-CH2-Ar).

Synthesis of [(SC4A)(D03A)(Eu(H2O)4)Na(H2O).17(H2O)] [3]
D03A (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%). Found: C, 35.02; H, 4.81; N: 3.92; S: 8.78. Calcd. for C34H34EuNaO22S6: C, 34.95; H, 4.68; N, 3.88; S, 8.89%. δH NMR (400 MHz, D2O) δ = 7.46 (s, 8 H, Ar-H), 3.91 (s, 8 H, Ar-CH2-Ar), 3.73, 2.94 (t, 2 H, N-CH2-CH2-N), 2.38 (s, 6 H, N-CH2-COOH), 2.28 (m, 2 H, N-CH2-CH2-N), 2.04 (m, 2 H, N-CH2-CH2-N), 1.90 (m, 2 H, N-CH2-CH2-N), 1.59 (m, 2 H, N-CH2-CH2-N).

Synthesis of [(SC4A)(Eu(H2O)4)]2 [5]
p-Sulfonatocalix[4]arene (0.5 g, 0.67 mmol) was dissolved in water (10 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 C34H13GdO12S13·48H2O: C, 29.49; H, 3.20; S, 11.60%. δH NMR (400 MHz, D2O) δ = 5.60 (br s, 8 H, Ar-H), 2.86 (br s, 8 H, Ar-CH2-Ar).

J. Name., 2013, 00, 1-3 | 10
10.66%, $\gamma_{\text{max}}$=1.0 cm$^{-1}$, 3248 (w, br) H$_2$O, 1155 (s) SO$_2$, 1119 (s), 1040 (s) SO$_4$. $m/z$ 1035.3403 (SC4A-Eu(OH)$_3$)]. 1H NMR (400 MHz, D$_2$O) $\delta$ 7.52 (s, 8 H, Ar-H), 3.78 (s, 8 H, Ar-CH$_2$-Ar).

**Synthesis of** 
[(Tb(H$_2$O)$_3$)(SC4A)(Tb(H$_2$O)$_3$)(SC4A)(Tb(H$_2$O)$_3$)(DO3A).12(H$_2$O)]$_n$ [6]

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added $p-$ sulphonatocalix[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 (274 mg, 39.2%). Found: C, 30.01; H, 5.23; S, 10.03. Calcd. for C$_{42}$H$_{114}$O$_{17}$S$_4$Gd$_3$(H$_2$O)$_4$: C, 25.94; H, 5.13; S, 9.89% $\gamma_{\text{max}}$=1.0 cm$^{-1}$ 3201 (w, br) H$_2$O, 1149 (s) SO$_2$, 1116 (s), 1037 (s) SO$_3$H. $m/z$ 1035.3416 (SC4A-Gd(OH)$_3$).

**Synthesis of** 
[(SC4A)(Eu(H$_2$O)$_3$)(SC4A)(Eu(H$_2$O)$_3$)(DO3A).19(H$_2$O)]$_n$ [7]

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with gadolinium acetate (0.23 g, 0.67 mmol) at 70°C. 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, 3.74; S, 12.20. Calcd. for C$_{70}$H$_{122}$N$_4$O$_{16}$S$_4$Gd$_3$: C, 30.33; H, 4.44; N, 2.02; S, 9.25%, $\gamma_{\text{max}}$=1.0 cm$^{-1}$ 3217 (w, br) H$_2$O, 1148 (s) SO$_2$, 1116 (s), 1037 (s) SO$_3$H. $m/z$ 1035.3402 (SC4A-Gd(OH)$_3$).

**Synthesis of** 
[(SC4A)(Eu(H$_2$O)$_3$)(SC4A)(Eu(H$_2$O)$_3$)(SC4A).35(H$_2$O)]$_n$ [8]

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added $p-$ sulphonatocalix[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 (3248 mg, 26.1%). Found: C, 32.25; H, 3.74; S, 12.20. Calcd. for C$_{110}$H$_{216}$N$_4$O$_{36}$S$_4$Eu$_3$: C, 32.37; H, 3.69; S, 12.35%, $\gamma_{\text{max}}$=1.0 cm$^{-1}$ 3232 (w, br) H$_2$O, 1150 (s) SO$_2$, 1117 (s), 1038 (s) SO$_3$H. $m/z$ 1035.3408 (SC4A-Eu(OH)$_3$)(H$_2$O)$_3$. 1H NMR (400 MHz, D$_2$O) $\delta$ 7.39 (s, 8 H, Ar-H), 3.97 (s, 8 H, Ar-CH$_2$-Ar).

**Synthesis of** 
[(SC4A)(Gd(H$_2$O)$_3$)(SC4A)(Gd(H$_2$O)$_3$)(SC4A).44(H$_2$O)]$_n$ [9]

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added $p-$ sulphonatocalix[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 C$_{42}$H$_{114}$O$_{17}$S$_4$Gd$_3$(H$_2$O)$_4$: C, 25.94; H, 5.13; S, 9.89% $\gamma_{\text{max}}$=1.0 cm$^{-1}$ 3201 (w, br) H$_2$O, 1149 (s) SO$_2$, 1116 (s), 1037 (s) SO$_3$H. $m/z$ 1035.3416 (SC4A-Gd(OH)$_3$).

**Synthesis of** 
[(SC4A)(Tb(H$_2$O)$_3$)(SC4A)(Tb(H$_2$O)$_3$)(SC4A).31(H$_2$O)]$_n$ [10]

DO3A (0.23 g, 0.67 mmol) was dissolved in water (10 mL) with stirring at 70°C. To this solution was added $p-$ sulphonatocalix[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 C$_{42}$H$_{114}$O$_{17}$S$_4$Tb$_3$: C, 32.16; H, 3.66; S, 12.26% $\gamma_{\text{max}}$=1.0 cm$^{-1}$ 3201 (w, br) H$_2$O, 1149 (s) SO$_2$, 1117 (s), 1038 (s) SO$_3$H. $m/z$ 1035.3404 (SC4A-Tb(H$_2$O)$_3$)(SC4A-Tb(H$_2$O)$_3$).

**Crystal structure determinations**

Crystals suitable for X-ray diffraction were grown by slow 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 $\omega$ scans using a STOE IPSPD2 image plate diffractometer utilising monochromated molybdenum radiation ($\lambda = 0.71073$ Å). Standard procedures were employed for the integration and processing of the data using X-RED.39 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 $F^2$ values using SHELX-97.30

For (2), data were collected by the EPSRC National Crystallography Service3 with 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 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.

Acknowledgements

We would like to thank the EPSRC UK National Crystallography Service at the University of Southampton for the collection of data. 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.

1 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.

2 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.