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Drug delivery of lipophilic pyrenyl derivatives by encapsulation in a water soluble metalla-cage

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The self-assembly of 2,4,6-tris(pyridin-4-yl)-1,3,5-triazine (tpt) triangular panels with *p*-cymene (*p*-Pr^{i}C$_6$H$_4$Me) ruthenium building blocks and 2,5-dioxydo-1,4-benzoquinonato (dobq) bridges, in the presence of a functionalised pyrenyl derivative (pyrene–R), affords the triangular prismatic host–guest compounds [(pyrene–R) ⊂ Ru$_6$(*p*-Pr^{i}C$_6$H$_4$Me)$_6$(tpt)$_2$(dobq)$_3$]$^{6+}$ ([(pyrene–R) ⊂ **1**]$^{6+}$). The inclusion of eight mono-substituted pyrenyl derivatives including biologically relevant structures (**a** = 1-pyrenebutyric acid, **b** = 1-pyrenebutanol, **c** = 1-pyrenemethylamine, **d** = 1-pyrenemethylbutanoate, **e** = 1-(4,6-dichloro-1,3,5-triazin-2-yl)pyrene, **f** = *N*-hexadecylpyrene-1-sulfonamide, **g** = pyrenyl ethacrynic amide and **h** = 2-(pyren-1-ylmethylcarbamoyl) phenyl acetate), and a di-substituted pyrenyl derivative (**i** = 1,8-bis(3-methyl-butyn-1-yl-3-ol)pyrene), has been accomplished. The carceplex nature of these systems with the pyrenyl moiety being firmly encapsulated in the hydrophobic cavity of the cage with the functional groups pointing outwards was confirmed by NMR (^{1}H, 2D, DOSY) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). The cytotoxicities of these water-soluble compounds have been established using human ovarian A2780 cancer cells. All the host–guest systems are more cytotoxic than the empty cage itself [**1**][CF$_3$SO$_3$]$_6$ (IC$_{50}$ = 23 μM), the most active carceplex [**f** ⊂ **1**][CF$_3$SO$_3$]$_6$ is an order of magnitude more cytotoxic.

Introduction

Since the discovery of the antiproliferative properties of cisplatin in the 1960s by Rosenberg,[1] platinum compounds have become the most widely used drugs in the treatment of cancer.[2] Although effective, cisplatin has severe side effects including nephrotoxicity and neurotoxicity due to low selectivity towards cancer cells.[3] Increasing the selectivity of cisplatin is a major challenge that has been addressed in numerous ways.[4] In order to achieve increased selectivity, *i.e.* increasing the amount of drug that reaches the tumour relative to the healthy tissue, drugs may be combined with a transport vector that accumulates in cancer cells.[5] In the case of platinum-based drugs, this strategy has been attempted by incorporating them into various structures, including carbon nanotubes,[6] proteins,[7] macrocycles[8] and dendrimers.[9] These macromolecular systems selectively accumulate in tumours by targeting the enhanced permeability and retention (EPR) effect.[10] This effect arises from increased angiogenesis and permeability mediator production combined with decreased lymphatic drainage, thus allowing the accumulation of macromolecules in cancer cells.

Ruthenium-based drugs have also been extensively explored in the treatment of cancer.[11] The potential clinical applications of ruthenium complexes, such as [ImH][*trans*-Ru(Im)$_2$Cl$_4$] (KP1019, Im = imidazole)[12] and [ImH][*trans*-Ru(DMSO)(Im)Cl$_4$] (NAMI-A, Im = imidazole, DMSO = dimethylsulfoxide)[13] as anticancer and antimetastatic agents has generated considerable interest. Recently, arene ruthenium complexes have started to attract a lot of attention and many show promising anticancer activity.[14] Arene ruthenium and other half sandwich complexes have also been used as supramolecular building blocks,[15] in which three coordination sites are occupied by the arene ligand, thus leaving only three open sites for further coordination. Moreover, the relative inertness of the arene ligand and the easy access to starting materials are appealing. When these complexes are used as supramolecular building blocks, structures with diverse functionalities and properties are obtained. Recently, we combined these two areas, *i.e.* the medicinal properties of arene ruthenium complexes and supramolecular arene ruthenium chemistry, to afford a new hybrid drug delivery system. The hexacationic prism [Ru$_6$(*p*-^{i}PrC$_6$H$_4$Me)$_6$(tpt)$_2$(dobq)$_3$]$^{6+}$ ([**1**]$^{6+}$) (tpt = 2,4,6-tris(pyridin-4-yl)-1,3,5-triazine; dobq = 2,5-dioxydo-1,4-benzoquinonato) was synthesized, which was capable of encapsulating planar Pt and Pd acetylacetonate complexes,[16] as well as planar aromatic compounds of various sizes (Chart 1).[17,18] In these systems the encapsulated guest is stable, with the physical properties of the prism being retained following encapsulation. The biological activity of some of these carceplexes has been evaluated and encouraging results were obtained. The metallaprism itself exhibits some activity, which increases with the encapsulation of a guest, suggesting transport and leaching of the guest once inside the cell.[16] The ability of [**1**]$^{6+}$ to deliver guest molecules to cells was confirmed by encapsulation of a fluorescently labelled pyrene–R derivative (1-(4,6-dichloro-1,3,5-triazin-2-yl)pyrene), and fluorescence spectroscopy was used to

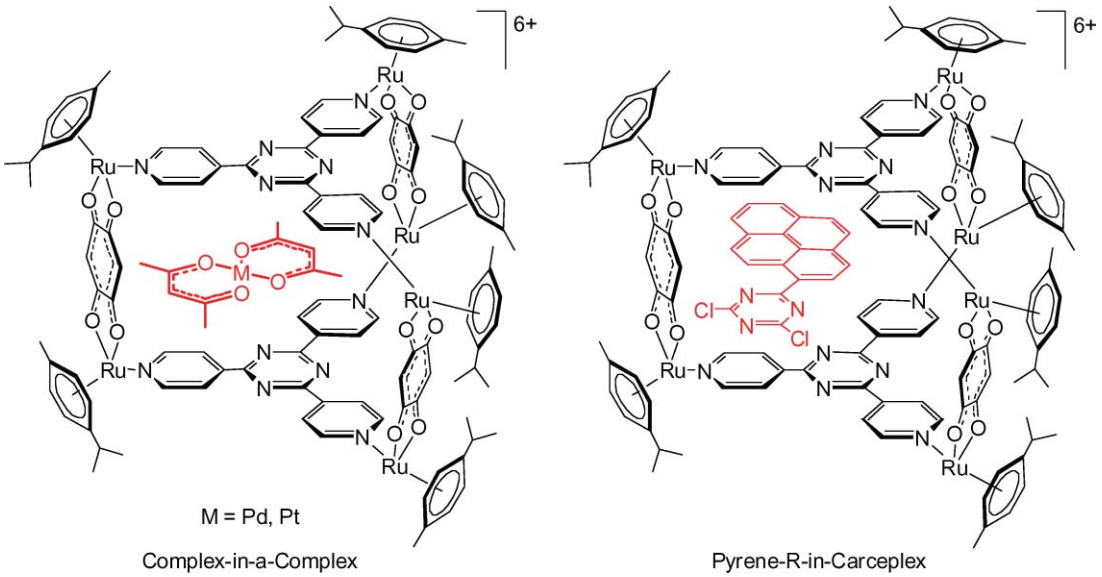


Chart 1

monitor the uptake and release of the pyrene–R molecule in cancer cells.[19] We have also previously shown that encapsulation is possible even with a pyrenyl derivative possessing pendant groups that extend beyond the interior of the cage.[17]

Herein, we describe the synthesis and characterisation of a series of pyrenyl derivatives possessing various functional groups, including biologically-active functionalities, and their encapsulation in the metalla-prism $[Ru_6(p\text{-}^{i}PrC_6H_4Me)_6(tpt)_2(dobq)_3]^{6+}$ ($[\mathbf{1}]^{6+}$). The *in vitro* anticancer activity of these water soluble systems was evaluated against human ovarian A2780 cancer cells and the implication of the metalla-prism to deliver hydrophobic organic drugs to cancer cells is discussed.

Results and discussion

A series of functionalised pyrenyl compounds, *i.e.* **a** = 1-pyrenebutyric acid, **b** = 1-pyrenebutanol, **c** = 1-pyrenemethylamine, **d** = 1-pyrenemethylbutanoate, **e** = 1-(4,6-dichloro-1,3,5-triazin-2-yl)pyrene and **f** = *N*-hexadecylpyrene-1-sulfonamide) and two new derivatives (**g** = pyrenyl ethacrynic amide and **h** = 2-(pyren-1-ylmethylcarbamoyl) phenyl acetate), see Chart 2, were encapsulated in the metalla-prism $[\mathbf{1}]^{6+}$. Most of the pyrenyl compounds are known compounds, whereas **g** and **h** are new compounds and were obtained from the reaction of 1-pyrenemethylamine and the corresponding acid chloride of ethacrynic acid and aspirin, respectively, which, in turn, are prepared, *in situ* from oxalyl chloride according to the literature method (see Experimental).[20] Three of the pyrenyl derivatives, **f**, **g** and **h**, contain bioactive functionalities (see below).

The encapsulation of the pyrenyl derivatives **a**–**h** in the metalla-prism $[\mathbf{1}]^{6+}$ is achieved in a two step process, in which the *p*-cymene ruthenium dobq (2,5-dioxydo-1,4-benzoquinonato) bimetallic "molecular clip" is first reacted with silver triflate to produce a reactive intermediate, which is then combined with

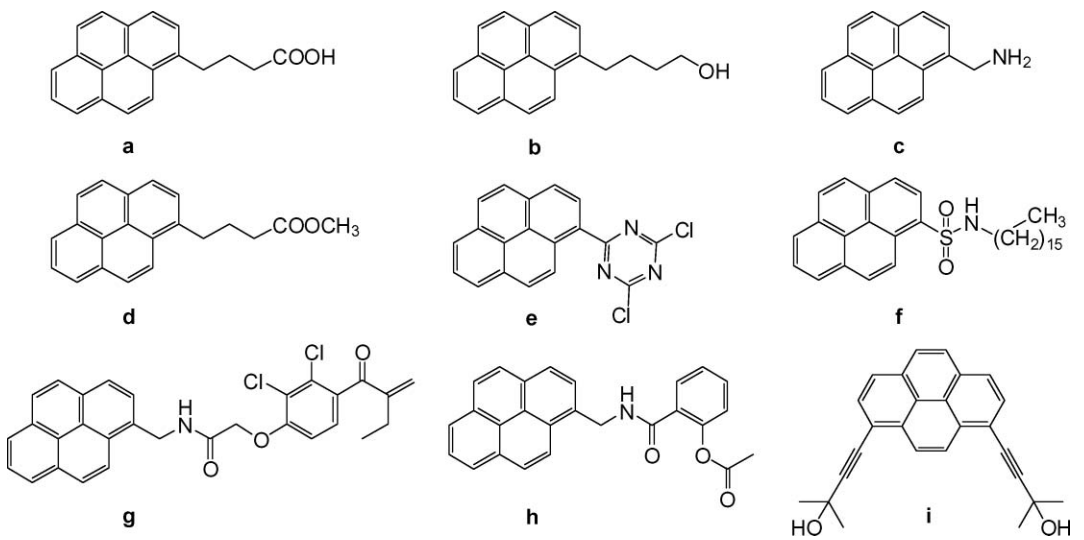
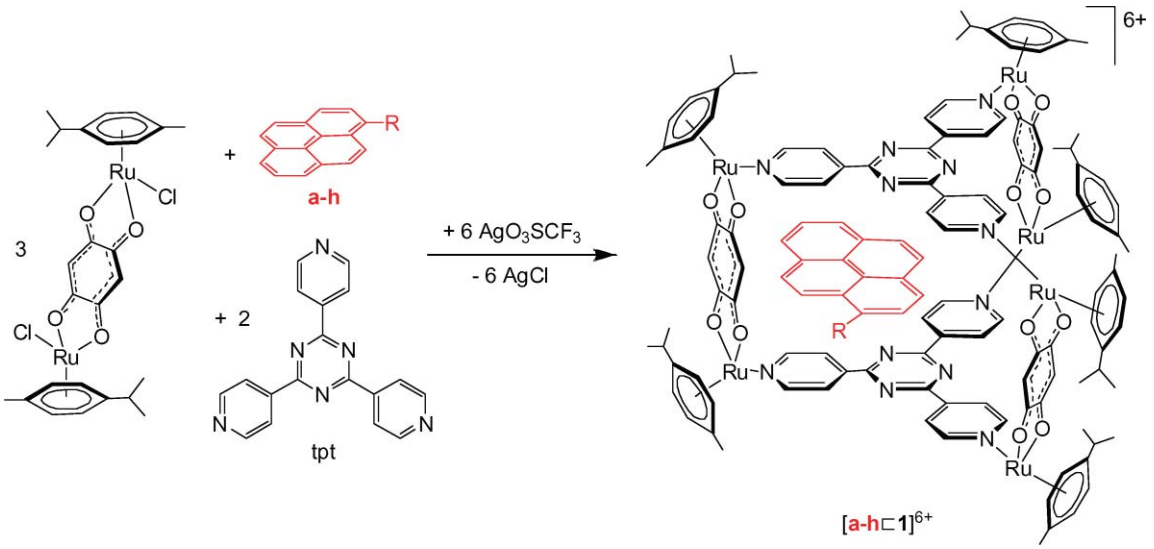


Chart 2



Scheme 1 The synthesis of the metalla-prisms [**a–h** ⊂ **1**][CF_3SO_3]$_6$ encapsulating a functionalised pyrenyl derivative.

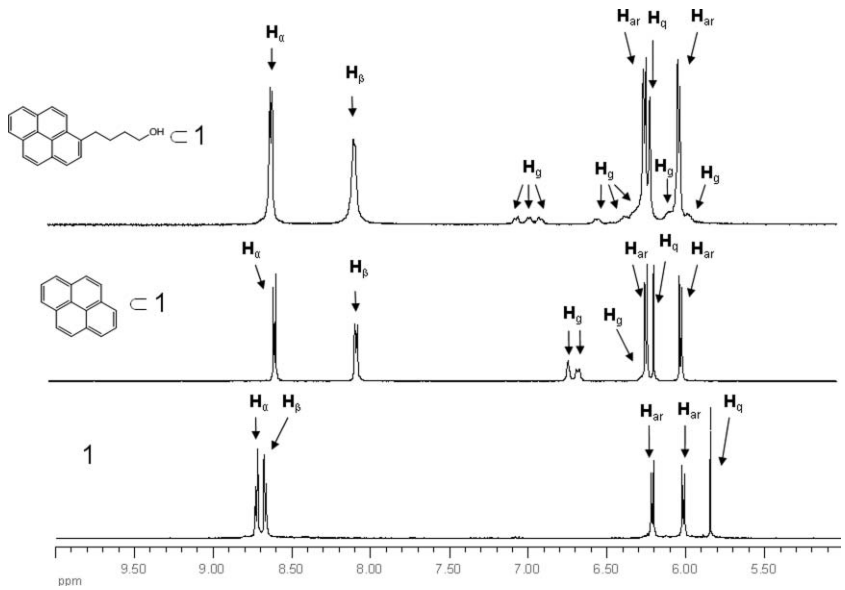
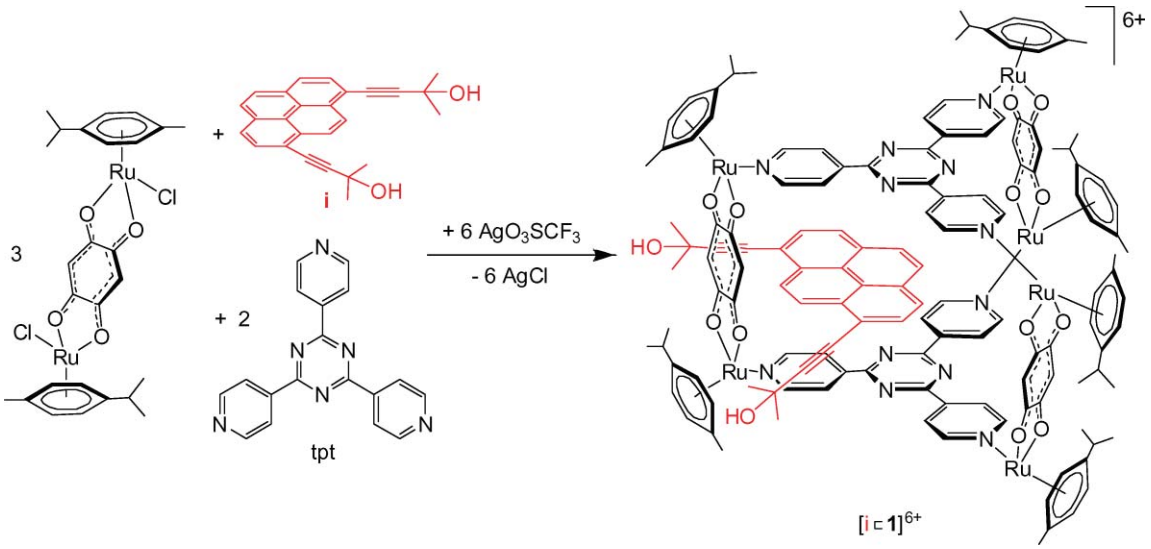


Fig. 1 The aromatic region of the ^{1}H NMR spectra (acetone-d_6) of [**1**]$^{6+}$ (bottom), [pyrene ⊂ **1**]$^{6+}$ (middle) and [**b** ⊂ **1**]$^{6+}$ (top).

a 2 : 1 mixture of tpt (2,4,6-tris(pyridin-4-yl)-1,3,5-triazine) and the functionalised pyrenyl derivative (Scheme 1), affording the corresponding inclusion system [**a–h** ⊂ **1**]$^{6+}$. All the inclusion complexes were isolated in good yield as their triflate salts.

The encapsulation process may be monitored by ^{1}H NMR spectroscopy; in acetone-d_6 a doublet corresponding to the H_β protons of the tpt panels appears at δ = 8.71 ppm in the empty cage [**1**]$^{6+}$,[16] and both broadens and shifts upfield by *ca.* 0.6 ppm upon encapsulation of the functionalised pyrenyl derivative or even pyrene (Fig. 1). Similarly, the nine signals of the pyrenyl group or the ten signals of pyrene are shifted upfield following encapsulation, which is as expected since the pyrenyl moiety is sandwiched between the two tpt panels *via* a π-stacking arrangement. In contrast, the signals of the dobq protons are shifted downfield in all systems and by as much as 0.4 ppm in the case of [**a** ⊂ **1**]$^{6+}$. In general, the signals of the adjacent methylene group of the pyrenyl side-chain are shifted downfield, whereas the rest of the signals associated to the side-chain remain virtually unchanged. To further confirm the encapsulation of the functionalised pyrenyl derivatives in the cavity of [**1**]$^{6+}$, a series of diffusion-ordered (DOSY) NMR spectra were recorded.

DOSY is a powerful tool for studying host–guest associations in solution.[21] The diffusion coefficient depends on the shape and size of the molecules. Therefore, in a carceplex system in which the guest is perfectly trapped in the cavity of the host, without significantly affecting the size and shape of the host, the diffusion coefficient of the guest ⊂ host adduct should be almost identical to the diffusion coefficient of the host alone. DOSY measurements of pyrenyl derivative **g**, the empty cage [**1**][CF_3SO_3]$_6$, and the inclusion system [**g** ⊂ **1**][CF_3SO_3]$_6$, are presented in Fig. 2. These experiments show that in [**g** ⊂ **1**]$^{6+}$, both components possess the same diffusion coefficient, which is almost identical to the diffusion coefficient of the empty cage [**1**]$^{6+}$, thus confirming the encapsulation of **g** in [**1**]$^{6+}$.



Scheme 2 The synthesis of carceplex $[\mathbf{i} \subset \mathbf{1}][CF_3SO_3]_6$.

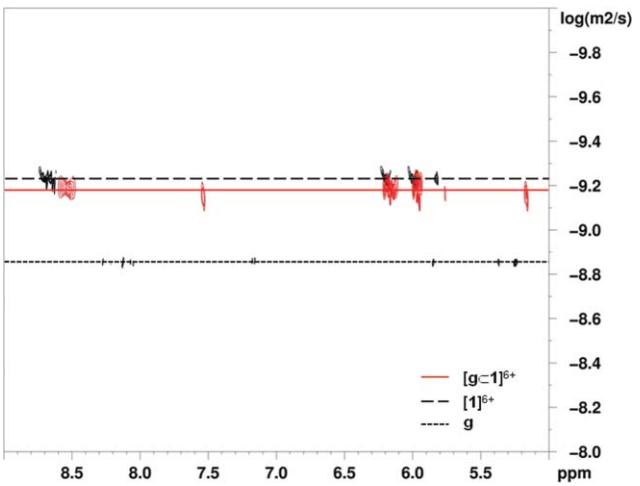


Fig. 2 The DOSY NMR spectra of **g**, $[\mathbf{1}]^{6+}$ and $[\mathbf{g} \subset \mathbf{1}]^{6+}$ in acetone-d_6 (24 °C).

Cage $[\mathbf{1}]^{6+}$ can also encapsulate di-functionalised pyrenyl derivatives, such as **i**. If one equivalent of 1,8-bis(3-methyl-butyn-1-yl-3-ol)pyrene, prepared using a published procedure,[22] is added to the reaction mixture, the carceplex $[\mathbf{i} \subset \mathbf{1}]^{6+}$ is obtained in good yield (Scheme 2). This structure is confirmed by ^{1}H NMR in which the characteristic broadening and shift of the H_β proton signal of the tpt panels is observed. For $[\mathbf{i} \subset \mathbf{1}]^{6+}$, however, two independent signals are observed for the dobq protons, which is in accordance with the symmetry and the deshielding effect produced by the proximity of the alkynyl bonds on four dobq protons (δ = 6.38 ppm), with the other two protons appearing at δ = 6.21 ppm. This carceplex system was further confirmed by electrospray ionization mass spectroscopy (ESI-MS) with the mass spectrum-containing peaks corresponding to $[\mathbf{i} \subset \mathbf{1} + (CF_3SO_3)_4]^{2+}$ at m/z = 1706.64 and to $[\mathbf{i} \subset \mathbf{1} + (CF_3SO_3)_3]^{3+}$ at m/z = 1088.84.

ESI-MS of all the carceplex systems corroborates their proposed structures; see Fig. 3 for selected examples. The ESI mass spectra of $[\mathbf{a–g} \subset \mathbf{1}][CF_3SO_3]_6$ show peaks corresponding to the cationic carceplex system with the loss of 2, 3 or 4 triflate counter ions.

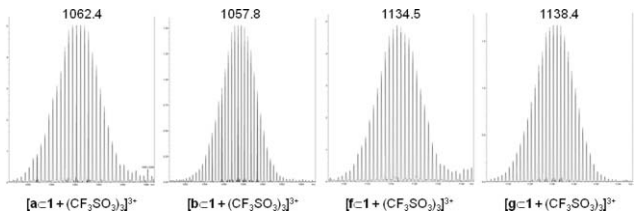


Fig. 3 Selected ESI-MS peaks of some carceplexes.

In the case of $[\mathbf{h} \subset \mathbf{1}]^{6+}$ the acetyl group of the aspirin moiety is cleaved. These peaks were assigned unambiguously on the basis of their characteristic isotope patterns. Moreover, a similar behaviour was observed under ESI-MS conditions with planar aromatic molecules encapsulated in $[\mathbf{1}]^{6+}$.[17]

The antiproliferative activity of $[\mathbf{1}]^{6+}$ and all the inclusion systems $[\mathbf{a–i} \subset \mathbf{1}][CF_3SO_3]_6$ was evaluated against the human ovarian A2780 cancer cell line using the MTT assay, which measures mitochondrial dehydrogenase activity as an indication of cell viability. The IC_{50} values are listed in Table 1 and are reported together with that of cisplatin for comparison purposes. As mentioned above, there is considerable on-going interest in the anticancer properties of arene ruthenium complexes.[14] It should be noted that some pyrenyl derivatives have recently been shown to interact with various DNA and RNA polynucleotides and have been tentatively proposed to have potential applications in the treatment of certain types of tumors.[23] However, pyrene-containing compounds are usually used as cellular probes due to

Table 1 The IC_{50} values determined on A2780 human ovarian cancer cells

Compound	IC_{50}/μM	Compound	IC_{50}/μM
$[\mathbf{1}]^{6+}$	23 ± 2	cisplatin	1.6
[pyrene ⊂ **1**]$^{6+}$	9 ± 2	$[\mathbf{e} \subset \mathbf{1}]^{6+}$	6 ± 1.5
$[\mathbf{a} \subset \mathbf{1}]^{6+}$	18 ± 1.5	$[\mathbf{f} \subset \mathbf{1}]^{6+}$	2 ± 0.6
$[\mathbf{b} \subset \mathbf{1}]^{6+}$	21 ± 2	$[\mathbf{g} \subset \mathbf{1}]^{6+}$	3 ± 1.1
$[\mathbf{c} \subset \mathbf{1}]^{6+}$	14 ± 2	$[\mathbf{h} \subset \mathbf{1}]^{6+}$	5 ± 0.4
$[\mathbf{d} \subset \mathbf{1}]^{6+}$	17 ± 1	$[\mathbf{i} \subset \mathbf{1}]^{6+}$	5 ± 0.8

their favourable fluorescence properties.[24] Here, however, due to the encapsulation of the pyrene moiety into the water soluble metalla-prism, the cytotoxic effect of even very hydrophobic derivatives can be assessed.

The empty cage, $[\mathbf{1}]^{6+}$, exhibits a moderate cytotoxicity of 23 μM, which is comparable to that of related supramolecular arene ruthenium and arene osmium cages carrying relatively high charges.[16,17,25] It should be noted that highly charged complexes cross cell membranes equally as well as neutral complexes, and in some cases, their ability to enter cells is superior to that of neutral compounds or compounds in a low charge state.[26] Encapsulation of the pyrenyl systems into the hexaruthenium cage has either a negligible effect on the cytotoxicity (pyrenes **a–d**) or significantly increases the cytotoxicity (pyrenes **e–i**), with $[\mathbf{f} \subset \mathbf{1}]^{6+}$ and $[\mathbf{g} \subset \mathbf{1}]^{6+}$ being an order of magnitude more cytotoxic than the empty cage $[\mathbf{1}]^{6+}$. Indeed, the cytotoxicity of $[\mathbf{f} \subset \mathbf{1}]^{6+}$ and $[\mathbf{g} \subset \mathbf{1}]^{6+}$ is comparable to cisplatin. These differences could be due to the intrinsic cytotoxicities of the different pyrenyl derivatives, which, due to the poor water solubility of these compounds, could not be evaluated, or to differences in the uptake and/or further intracellular release of these molecules.

The substituent tethered to the pyrenyl ring in $[\mathbf{f} \subset \mathbf{1}]^{6+}$, the most active compound of the series, contains a group that is not too dissimilar from an extensive range of sulfonamide-containing inhibitors of carbonic anhydrase.[27] Carbonic anhydrases represent potential targets for anticancer drugs[28] and therefore it is conceivable that the high cytotoxicity of $[\mathbf{f} \subset \mathbf{1}]^{6+}$ corresponds, at least in part, to the inhibition of this enzyme class.

The substituent tethered to the pyrenyl ring in $[\mathbf{g} \subset \mathbf{1}]^{6+}$, which is also a very cytotoxic compound, is ethacrynic acid, which is an excellent inhibitor of glutathione transferases and has even been investigated as a potential anticancer drug in a combination therapy with cisplatin.[29] Indeed, arene ruthenium compounds with tethered ethacrynic acid moieties are also good glutathione transferase inhibitors and are moderately cytotoxic,[30] and the crystal structure of an arene ruthenium compounds containing ethacrynic acid embedded in the active site of the human glutathione transferase P1-1 has been reported.[20] Glutathione transferases catalyze the nucleophilic attack by reduced glutathione (GSH) on non-polar nucleophiles, acting on a range of exogenous compounds, including anticancer agents, forming part of a coordinated defence strategy to remove GSH conjugates from the cell.[31] Consequently, inhibition of this enzyme means that an anticancer drug can function more effectively and it is possible that $[\mathbf{g} \subset \mathbf{1}]^{6+}$ exerts its cytotoxicity by the ethacrynic acid derivative inhibiting glutathione transferase within the cell resulting in a sensitized cell that is more responsive to the empty cage, $[\mathbf{1}]^{6+}$.

Conclusions

Targeting anticancer drugs to the tumour environment is an important goal in medicinal chemistry, especially for anticancer compounds that exert their antineoplastic effect *via* DNA but also for drugs that act on other targets. In addition, platinum anticancer drugs are usually administered in combination therapies with organic drugs and it could be advantageous to ensure that the drugs reach the tumour at the same time. Many different drug targeting strategies have been assessed[5b,32] and herein we describe a nascent class of drug delivery vectors based on the concepts of supramolecular chemistry.[16,19] Specifically, a series of hexaruthenium carceplex systems encapsulating functionalised pyrenyl derivatives have been prepared and characterized. The compounds are stable and water soluble and were evaluated *in vitro* for anticancer activity. The *in vitro* study reveals that the nature of the pyrenyl derivative strongly influences the cytotoxicity of the system, and consequently, by tuning the nature of the functional group attached on the pyrenyl unit, highly cytotoxic species can be produced, thus confirming that these metalla-cages offer a strategy to deliver drugs to cancer cells at the same time. The two most cytotoxic compounds contain a pyrenyl ring functionalised with a possible carbonic anhydrase inhibitor and a glutathione transferase inhibitor. Inhibition of both of these enzymes is useful in certain types of cancer, but since these enzymes are ubiquitous, selective delivery to the tumour environment is important in order to avoid additional side effects. Therefore, delivery within the supramolecular host molecule could prove advantageous, although it should be noted that an *in vivo* study is required to establish whether these systems do accumulate in the tumour. However, prior to an *in vivo* study further optimisation of the system is required in order to find the optimum system to evaluate.

Experimental

General details

All organic solvents were degassed and saturated with nitrogen prior to use. $[Ru_2(p\text{-}Pr^iC_6H_4Me)_2(dobq)Cl_2]$,[16] diiodo-pyrene[22] and 2,4,6-tris(4-pyridyl)-1,3,5-triazine (tpt)[33] were prepared according to published methods. All other reagents were commercially available and used as received. The ^1H, $^{13}C\{^1H\}$ and DOSY NMR spectra were recorded on a Bruker AvanceII 400 spectrometer using the residual protonated solvent as an internal standard.[34] Infrared spectra were recorded as KBr pellets on a Perkin-Elmer FTIR 1720X spectrometer. Electrospray ionization mass spectrometry were recorded on a Bruker APEX II 9.4-tesla FT-ICR-MS equipped with an Apollo II electrospray ion source; sample conditions: 10–50 μmol l^{-1} in methanol at 30 °C, end plate voltage 3500 V, and capillary voltage 4000 V. UV-visible absorption spectra were recorded on a Uvikon 930 spectrophotometer using precision cells made of quartz (1 cm).

Compound synthesis

Pyrenyl ethacrynic amide (g). Ethacrynic acid (0.23 g, 0.756 mmol) was suspended in oxalyl chloride (5 mL, 60 mmol) and refluxed for 1 h. The unreacted oxalyl chloride was removed under reduced pressure. A suspension of methylamine pyrene (0.202 g, 0.756 mmol) and Et_3N (0.75 mL, 5.3 mmol) in THF (50 mL) was added drop-wise to the ethacrynic acid chloride. The mixture was then stirred at 60 °C for 18 h. The solvent was removed under reduced pressure and the product re-dissolved in $CHCl_3$. The solution was filtered and the filtrate was washed with $NaHCO_3$ solution and thereafter brine, dried over $MgSO_4$ and the solvent evaporated to give an oil, which was purified on a silica gel column, mobile phase: dichloromethane : acetone 3 : 1, to give the product as a pale yellow powder. Yield: 163 mg (39%). ν_{max}/cm^{-1} 3427 (br), 3267(m), 1653(vs), 1586(m), 1564(m), 1466(m), 1382(w), 1251(m),

1124(w), 1089(m), 998(w), 849(s), 841(m), 827(w), 797(w), 714(w); δ_H (400 MHz; CD_2Cl_2) 8.32 (1H, d, *J* 9.24, H_{py}), 8.24 (2H, m, H_{py}), 8.20 (d, 1H, *J* 2.24, H_{py}), 8.18 (1H, s, H_{py}), 8.07 (4H, m, H_{py}), 7.18 (1H, s, NH), 7.12 (1H, d, *J* 8.52, H_{Ar}), 6.89 (d, 1H, *J* 8.55, H_{Ar}), 5.85 (1H, t, *J* 1.38, Hc = *c*), 5.42 (1H, s, Hc = *c*), 5.28 (2H, d, *J* 5.80, Py–*CH*$_2$–NH), 4.68 (2H, s, CO–*CH*$_2$–O), 2.40 (2H, q, *J* 7.41, C–*CH*$_2$–CH$_3$), 1.10 (3H, t, *J* 7.44, CH_3); δ_C (100 MHz; CD_2Cl_2) 206.50, 195.36, 166.47, 154.69, 150.18, 134.19, 131.40, 131.33, 131.00, 130.84, 128.93, 128.68, 128.27, 127.61, 127.44, 127.318, 126.88, 126.27, 125.56, 125.49, 125.04, 124.95, 124.73, 122.73, 111.34, 68.66, 41.36, 30.68, 23.43, 12.27; *m/z* (ESI) 553.6 [M (2 × ^{35}Cl) + K]$^+$, 555.6 [M (^{35}Cl, ^{37}Cl) + K]$^+$, 557.6 [M (2 × ^{37}Cl) + K]$^+$.

2-(Pyren-1-ylmethylcarbamoyl) phenyl acetate (h). Aspirin (150 mg, 0.83 mmol) was suspended in thionyl chloride (20 mL) and stirred for 4 h at room temperature. The thionyl chloride was removed under reduced pressure and then a mixture of 1-pyrenemethylamine (223 mg, 0.83 mmol) and Et_3N (0.12 mL, 0.85 mmol) in THF (50 mL) was slowly added. The mixture was stirred at 60 °C for 18 h. The solvent was removed under reduced pressure and the product re-dissolved in chloroform. The solution was filtered and the filtrate washed with $NaHCO_3$ solution and thereafter brine, dried over $MgSO_4$ and then the solvent evaporated to give an oil, which was purified on a silica gel column, mobile phase: dichloromethane : acetone 3 : 1, to give the product as a pale yellow powder. Yield: 50 mg (15%). ν_{max}/cm^{-1} 3332(w), 1635 (m), 1583 (s), 1537(s), 1493(m), 1443(w), 1356(s), 1300(m), 1246(m), 1229(m), 1216(s), 1033(w), 847(s), 817(w), 758(m), 721(w), 704(w); δ_H (400 MHz; CD_2Cl_2) 8.33 (1H, d, *J* 9.25, H_{py}), 8.21 (4H, m, H_{py}), 8.06 (4H, m, H_{py}), 7.38 (1H, ddd, *J* 7.81 and 1.52, H_{Ar}), 7.32 (1H, dd, *J* 8.02 and 1.51, H_{Ar}), 6.97 (1H, dd, *J* 8.38 and 1.02, H_{Ar}), 6.78 (1H, ddd, *J* 7.62 and 1.17, H_{Ar}), 6.75 (1H, br, NH), 5.35 (2H, d, *J* 5.37, Py–*CH*$_2$–NH), 1.27 (3H, s, CH_3); δ_C (100 MHz; CD_2Cl_2) 169.768, 161.849, 134.398, 131.502, 131.382, 130.847, 130.515, 129.200, 128.465, 127.747, 127.421, 127.311, 126.335, 125.623, 125.547, 125.079, 124.948, 124.698, 122.736, 118.695, 118.492, 114.235, 42.035, 29.796; *m/z* (ESI) 350.3 [(M–CH$_2$CO)–H]$^-$.

Synthesis of [a–h ⊂ 1][CF$_3$SO$_3$]$_6$. [Ru$_2$(*p*-Pr^{i}C$_6$H$_4$Me)$_2$-(dobq)Cl$_2$] (50 mg, 0.0736 mmol) and $AgCF_3SO_3$ (38 mg, 0.147 mmol) was stirred in methanol (30 mL) for 2 h, thereafter the solution was filtered into a suspension of tpt (15 mg, 0.049 mmol) and pyrenyl (1-pyrenebutanol 6.8 mg, 0.025 mmol; 1-pyrenemethyl butanoate 7.5 mg, 0.025 mmol; 1-pyrenebutyric acid 7.1 mg, 0.025 mmol; 1-pyrenemethylamine 6.7 mg, 0.025 mmol; *N*-hexadecylpyrene-1-sulfonamide 12.6 mg, 0.025 mmol; 1-(4,6-dichloro-1,3,5-triazin-2yl)pyrene 8.8 mg, 0.025 mmol; pyrenyl ethacrynic amide 12.7 mg, 0.025 mmol; 2-(pyren-1-ylmethylcarbamoyl) phenyl acetate 9.7 mg, 0.025 mmol) in methanol (10 mL). The mixture was stirred at RT for 18 h. The methanol was removed under reduced pressure and then the product was re-dissolved in CH_2Cl_2 before being filtered. The filtrate was reduced to about 5 ml and the product precipitated with diethyl ether and collected by filtration as a red powder.

*[**a** ⊂ **1**][CF$_3$SO$_3$]$_6$.* Yield: 68 mg (77%). λ_{max} (MeOH)/nm 495 (ε/dm^3 mol^{-1} cm^{-1} 46000), 351 (42000), 304 (81000), 281 (57000) and 271 (54000); ν_{max}/cm^{-1} 1524(s), 1377(s), 1258(s), 1224(w), 1159(w), 1030(m), 811(w), 638(w); δ_H(400 MHz; acetone-d_6) 8.58 (12H, d, *J* 6.39, H_α), 8.07 (12H, br, H_β), 7.24 (1H, d, *J* 4.43, H_g), 7.11 (1H, m, H_g), 6.99 (1H, br, H_g), 6.91 (1H, br, H_g), 6.57 (1H, br, H_g), 6.34 (1H, br, H_g), 6.22 (12H, d, *J* 6.24, Ar$_{p\text{-cym}}$), 6.18 (6H, s, H_q), 6.13 (2H, br, H_g), 6.00 (12H, d, Ar$_{p\text{-cym}}$), 5.85 (1H, br, H_g), 2.98 (6H, septet, *J* 6.91, CH$_{p\text{-cym}}$), 2.62 (2H, br, H_g), 2.50 (2H, br, H_g), 2.22 (18H, s, CH$_{3\ p\text{-cym}}$), 1.62 (2H, br, H_g), 1.39 (36H, d, CH$_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 184.32, 167.90?, 154.00, 143.48, 124.32, 104.21, 102.08, 99.37, 83.96, 82.37, 31.29, 21.71, 17.31; *m/z* (ESI) 1062.42 [**a** ⊂ **1** + (CF$_3$SO$_3$)$_3$]$^{3+}$, 759.32 [**a** ⊂ **1** + (CF$_3$SO$_3$)$_2$]$^{4+}$.

*[**b** ⊂ **1**][CF$_3$SO$_3$]$_6$.* Yield: 78 mg (88%). λ_{max} (MeOH)/nm 493 (ε/dm^3 mol^{-1} cm^{-1} 45000), 342 (47000), 308 (81000) and 275 (64000); ν_{max}/cm^{-1}1523(s), 1377(s), 1258(s), 1224(w), 1159(w), 1030(m), 811(w), 638(w); δ_H(400MHz; acetone-d_6) 8.58 (12H, d, *J* 6.4, H_α), 8.06 (12H, br, H_β), 7.03 (1H, d, *J* 8.8, H_g), 6.95 (1H, d, *J* 6.8, H_g), 6.87 (1H, d, *J* 8.4, H_g), 6.52 (1H, d, *J* 8.8, H_g), 6.22 (12H, d, *J* 6.4, Ar$_{p\text{-cym}}$), 6.18 (6H, s, H_q), 6.00 (12H, d, Ar$_{p\text{-cym}}$), 3.76 (2H, d, *J* 5.2, H_g), 2.98 (6H, septet, *J* 6.8, CH$_{p\text{-cym}}$), 2.52 (2H, t, *J* 7.2, H_g), 2.22 (18H, s, CH$_{3\ p\text{-cym}}$), 1.63 (2H, m, H_g), 1.47 (2H, m, H_g), 1.39 (36H, d, *J* 7.2, CH$_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 185.109, 168.581, 154.772, 144.277, 137.478, 130.689, 130.107, 128.943, 128.444, 128.038, 127.640, 127.134, 126.639, 126.587, 125.683, 125.395, 125.366, 125.143, 124.007, 123.808, 123.745, 123.200, 120.804, 104.986, 102.866, 100.193, 84.779, 83.140, 62.257, 33.71, 33.21, 32.09, 22.50, 18.11; *m/z* (ESI) 1057.79 [**b** ⊂ **1** + (CF$_3$SO$_3$)$_3$]$^{3+}$, 756.09 [**b** ⊂ **1** + (CF$_3$SO$_3$)$_2$]$^{4+}$.

*[**c** ⊂ **1**][CF$_3$SO$_3$]$_6$.* Yield: 49 mg (56%). λ_{max} (MeOH)/nm 494 (ε/dm^3 mol^{-1} cm^{-1} 43000), 341 (54000), 309 (83000) and 275 (73000); ν_{max}/cm^{-1} 1524(s), 1377(s), 1259(s), 1225(w), 1161(w), 1031(m), 812(w), 639(w); δ_H(400 MHz; acetone-d_6) 8.59 (12H, br, H_α), 8.22 (12H, br, H_β), 6.20 (12H, d, *J* 6.21, Ar$_{p\text{-cym}}$), 6.14 (6H, s, H_q), 5.99 (12H, d, Ar$_{p\text{-cym}}$), 2.98 (6H, septet, *J* 6.96, CH$_{p\text{-cym}}$), 2.22 (18H, s, CH$_{3\ p\text{-cym}}$), 1.40 (36H, d, CH$_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 185.23, 154.02, 124.40, 104.07, 101.90, 99.20, 83.83, 82.30, 31.36, 21.58, 17.27; *m/z* (ESI) 1043.41 [**c** ⊂ **1** + (CF$_3$SO$_3$)$_3$]$^{3+}$, 745.07 [**c** ⊂ **1** + (CF$_3$SO$_3$)$_2$]$^{4+}$.

*[**d** ⊂ **1**][CF$_3$SO$_3$]$_6$.* Yield: 64 mg (72%). λ_{max} (MeOH)/nm 494 (ε/dm^3 mol^{-1} cm^{-1} 46000) 342 (83800), 298 (113100), 275 (147200), 242 (260800) and 211 (274300); ν_{max}/cm^{-1} 1731(w), 1523(vs), 1376(s), 1258(s), 1159(w), 1057(w), 1030(m), 811(w), 638(m); δ_H(400 MHz; acetone-d_6) 8.58 (12H, d, *J* 6.54, H_α), 8.10 (12H, br, H_β), 7.14 (d, 1H, *J* 9.11, H_g) 6.98 (1H, d, *J* 7.42, H_g), 6.92 (1H, d, *J* 8.88, H_g), 6.33 (1H, dd, *J* 7.41, H_g), 6.26 (1H, br, H_g), 6.22 (12H, d, *J* 6.31, Ar$_{p\text{-cym}}$), 6.19 (1H, m, H_g) 6.16 (6H, s, H_q), 6.08 (1H, d, *J* 7.56, H_g), 6.00 (12H, d, *J* 6.31, Ar$_{p\text{-cym}}$), 5.85 (1H, d, *J* 7.53, H_g), 3.8 (3H, s, H_g), 2.98 (6H, septet, *J* 6.94, CH$_{p\text{-cym}}$), 2.54 (4H, m, H_g), 2.23 (18H, s, CH$_{3\ p\text{-cym}}$), 1.67 (2H, m, H_g), 1.40 (36H, d, *J* 6.94 Hz, CH$_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 185.19, 183.10, 168.83, 154.89, 144.39, 128.55, 127.87, 127.39, 126.73, 126.47, 125.89, 125.54, 125.25, 125.16, 124.11, 123.60, 123.18, 123.13, 120.91, 105.06, 102.93, 84.83, 83.30, 52.27, 34.26, 32.19, 26.89, 22.60, 18.22; *m/z* (ESI) 1067.10 [**d** ⊂ **1** + (CF$_3$SO$_3$)$_3$]$^{3+}$, 1674.70 [**d** ⊂ **1** + (CF$_3$SO$_3$)$_4$]$^{2+}$.

*[**e** ⊂ **1**][CF$_3$SO$_3$]$_6$.* Yield: 59 mg (65%). λ_{max} (MeOH)/nm 491 (ε/dm^3 mol^{-1} cm^{-1} 49000), 373 (51000), 300 (99000) and 271 (67000); ν_{max}/cm^{-1} 1523(s), 1377(s), 1258(s), 1224(w), 1159(w), 1030(m), 811(w), 638(w); δ_H(400 MHz; acetone-d_6) 8.54 (12H, br, H_α), 8.00 (12H, br, H_β), 6.20 (12H, d, *J* 6.02, Ar$_{p\text{-cym}}$), 6.18 (6H, s, H_q), 5.98 (12H, d, Ar$_{p\text{-cym}}$), 2.97 (6H, septet, *J* 6.68, CH$_{p\text{-cym}}$), 2.21 (18H, s, CH$_{3\ p\text{-cym}}$), 1.39 (36H, d, CH$_{3\ p\text{-cym}}$); δ_C (100 MHz;

acetone-d_6) 184.37, 154.07, 124.31, 104.25, 102.19, 99.34, 83.93, 82.37, 31.29, 21.69, 17.31; *m/z* (ESI-MS) 1081.40 [**e** ⊂ **1** + $(CF_3SO_3)_3]^{3+}$, 773.81 [**e** ⊂ **1** + $(CF_3SO_3)_2]^{4+}$.

*[**f** ⊂ **1**][CF_3SO_3]$_6$.* Yield: 61 mg (65%). λ_{max} (MeOH)/nm 492 (ε/dm^3 mol^{-1} cm^{-1} 39000), 351 (41000), 301 (76000) and 281 (66000); ν_{max}/cm^{-1} 1523(s), 1377(s), 1258(s), 1224(w), 1158(w), 1030(m), 811(w), 638(w); δ_H(400 MHz; acetone-d_6) 8.63 (12H, br, H_α), 8.42 (12H, br, H_β), 6.22 (12H, d, *J* 6.46, $Ar_{p\text{-cym}}$), 6.02 (6H, s, H_q), 6.01 (12H, d, $Ar_{p\text{-cym}}$), 2.99 (6H, septet, *J* 6.93, $CH_{p\text{-cym}}$), 2.25 (18H, s, $CH_{3\ p\text{-cym}}$), 1.40 (36H, d, $CH_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 184.32, 154.32, 124.81, 104.19, 101.96, 99.32, 83.94, 82.42, 31.44, 21.75, 17.53; *m/z* (ESI)1134.48 [**f** ⊂ **1** + $(CF_3SO_3)_3]^{3+}$, 813.63 [**f** ⊂ **1** + $(CF_3SO_3)_2]^{4+}$.

*[**g** ⊂ **1**][CF_3SO_3]$_6$.* Yield: 75 mg (78%). λ_{max} (MeOH)/nm 492 (ε/dm^3 mol^{-1} cm^{-1} 45000), 341 (56000), 309 (84000) and 275 (76000); ν_{max}/cm^{-1} 1524(s), 1377(s), 1259(s), 1225(w), 1159(w), 1031(m), 811(w), 638(w); δ_H(400 MHz; acetone-d_6) 8.56 (12H, br, H_α), 8.36 (2H, br, H_g), 8.00 (12H, br, H_β), 7.56 (2H, br, H_g), 7.23 (1H, br, H_g), 7.09 (1H, br, H_g), 6.80 (1H, br, H_g), 6.20 (12H, d, *J* 6.08, $Ar_{p\text{-cym}}$), 6.18 (6H, s, H_q), 6.05 (2H, br, H_g), 5.99 (12H, d, $Ar_{p\text{-cym}}$), 5.83 (2H, br, H_g), 5.79 (2H, br, H_g), 5.18 (2H, s, H_g), 4.52 (2H, br, H_g), 2.98 (6H, septet, *J* 6.93, $CH_{p\text{-cym}}$), 2.53 (2H, q, *J* 7.07, H_g), 2.23 (18H, s, $CH_{3\ p\text{-cym}}$), 1.39 (36H, d, $CH_{3\ p\text{-cym}}$), 1.21 (3H, t, H_g); δ_C (100 MHz; acetone-d_6) 184.27, 167.55, 153.96, 124.34, 104.21, 102.03, 99.24, 83.91, 82.45, 31.30, 21.71, 17.34. *m/z* (ESI) 1138.42 [**g** ⊂ **1** + $(CF_3SO_3)_3]^{3+}$, 816.32 [**g** ⊂ **1** + $(CF_3SO_3)_2]^{4+}$.

*[**h** ⊂ **1**][CF_3SO_3]$_6$.* Yield: 66 mg (72%). λ_{max} (MeOH)/nm 495 (ε/dm^3 mol^{-1} cm^{-1} 43000), 350 (46000), 342 (46000), 306 (81000), 281 (59000) and 276 (58000); ν_{max}/cm^{-1} 1524(s), 1377(s), 1258(s), 1224(w), 1158(w), 1030(m), 811(w), 637(w); δ_H(400 MHz; acetone-d_6) 9.12 (1H, br, H_g), 8.56 (12H, br, H_α), 7.97 (12H, br, H_β), 7.73 (2H, br, H_g), 7.39 (2H, br, H_g), 7.25 (1H, br, H_g), 6.95 (2H, br, H_g), 6.19 (19H, br, $Ar_{p\text{-cym}}$, Hq, H_g), 5.99 (13H, br, $Ar_{p\text{-cym}}$, H_g), 4.79 (2H, br, H_g), 2.98 (6H, septet, *J* 6.64, $CH_{p\text{-cym}}$), 2.26 (18H, s, $CH_{3\ p\text{-cym}}$), 1.41 (36H, d, $CH_{3\ p\text{-cym}}$); δ_C(100 MHz; acetone-d_6) 184.23, 167.59, 153.91, 124.31, 104.12, 101.93, 83.91, 82.54, 65.31, 31.30, 21.69, 17.33, 14.81. *m/z* (ESI) 1083.46 [**h** ⊂ **1** - CH_3CO + $(CF_3SO_3)_3]^{3+}$, 775.34 [**h** ⊂ **1** – CH_3CO + $(CF_3SO_3)_2]^{4+}$.

1,8-Bis(3-methyl-butyn-1-yl-3-ol)pyrene. A Schlenk flask was charged with a solution of 2-methyl-but-3-yn-2-ol (1 mL, 10.3 mmol) in freshly distilled diethylamine (60 mL). The solution was freeze-pump-thaw degassed and transferred to a mixture of 1,6- and 1,8-diiodo pyrene (2.0 g, 4.4 mmol), $Pd[PPh_3]_2Cl_2$ (68 mg), and CuI (0.12 mmol) under nitrogen atmosphere. The reaction mixture was heated to 50 °C under nitrogen for 20 h. The solvent was removed under vacuum and the product was dissolved in dichloromethane and filtered. The filtrate was then evaporated and the crude product was purified by column chromatography, eluted with dichloromethane–methanol (1% methanol). The product was obtained as a yellow solid (0.1 g, Yield 6%). δ_H (400 MHz; $CDCl_3$) 8.60 (2H, s), 8.10 (4H, s), 8.04 (2H, s), 1.81 (12H, s).

The encapsulation of **i** in [**1**]$^{6+}$ follows the same procedure as for [**a–h** ⊂ **1**][CF_3SO_3]$_6$.

*[**i** ⊂ **1**][SO_3CF_3]$_6$.* Yield 78 mg (85%). λ_{max}(MeOH)/nm 491 (ε/dm^3 mol^{-1} cm^{-1} 51000), 383 (90000), 362 (69000), 345 (47000), 301 (88000), 290 (106000) and 278 (72000); ν_{max}/cm^{-1} 1524(s), 1377(s), 1259(s), 1161(w), 1030(m), 811(w), 638(w); δ_H (400 MHz; acetone-d_6) 8.56 (12H, br, H_α), 8.03 (12H, br, H_β), 7.08 (1H, br, H_g), 6.79 (2H, br, H_g), 6.38 (4H, br, H_q), 6.21 (14H, m, $Ar_{p\text{-cym}}$, H_q), 6.06 (1H, br, H_g), 5.99 (12H, d, $Ar_{p\text{-cym}}$), 5.80 (2H, br, H_g), 5.54 (1H, br, H_g), 5.06 (2H, br, H_g), 2.98 (6H, septet, *J* 6.89, $CH_{p\text{-cym}}$), 2.21 (18H, s, $CH_{3\ p\text{-cym}}$), 1.74 (12H, s, CH_{3g}), 1.38 (36H, d, $CH_{3\ p\text{-cym}}$); δ_C (100 MHz; acetone-d_6) 185.03, 154.85, 125.15, 104.99, 100.23, 84.77, 83.04, 32.04, 22.48, 18.08; *m/z* (ESI) 1705.13 [**i** ⊂ **1** + $(CF_3SO_3)_4]^{2+}$, 1088.44 [**i** ⊂ **1** + $(CF_3SO_3)_3]^{3+}$.

Cell culture and inhibition of cell growth

Human A2780 ovarian carcinoma cells were obtained from the European Centre of Cell Cultures (ECACC, Salisbury, UK) and maintained in culture as described by the provider. The cells were routinely grown in RPMI 1640 medium with GlutaMAX™ containing 5% foetal calf serum (FCS) and antibiotics (penicillin and ciproxin) at 37 °C and 5% CO_2. For the evaluation of growth inhibition, the cells were seeded in 96-well plates (25×10^3 cells per well) and grown for 24 h in complete medium. Complexes were made up to the required concentration and added to the cell culture for 72 h incubation. Solutions of the compounds were applied by diluting a freshly prepared stock solution of the corresponding compound in aqueous RPMI medium with GlutaMAX™ (20 mM). Following drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.25 mg ml^{-1} and incubated for 2 h, then the culture medium was aspirated and the violet formazan (artificial chromogenic precipitate of the reduction of tetrazolium salts by dehydrogenases and reductases) dissolved in DMSO. The optical density of each well (96-well plates) was quantified three times in quadruplicates at 540 nm using a multiwell plate reader (iEMS Reader MF, Labsystems, US), and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} values for the inhibition of cell growth were determined by fitting the plot of the logarithmic percentage of surviving cells against the logarithm of the drug concentration using a linear regression function. The median value and the median absolute deviation were obtained from the Excel™ software (Microsoft™) and those values are reported in Table 1.

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