Supplementary Material (ESI) for Chemical Communications

Supporting Information

Hexanuclear Self-assembled Arene-Ruthenium Nano-Prismatic Cages: Potential Anticancer Agents

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General Details: Donor **1**[1] and arene-ruthenium acceptors [2] were prepared according to reported methods. Deuterated solvents were purchased from Cambridge Isotope Laboratory (Andover, MA). NMR spectra were recorded on either a Bruker 300 MHz spectrometer. ^{1}H NMR chemical shifts are reported relative to residual solvent signals. Mass spectra for the self-assemblies were recorded on a Micromass Quattro II triple-quadrupole mass spectrometer using electrospray ionization with a MassLynx operating system. [3] From a single crystal of **8**, the diffraction data were collected at 100 K on an ADSC Quantum 210 CCD diffractometer with synchrotron radiation (λ = 0.90000 Å) at the Macromolecular Crystallography Beamline 6B1, Pohang Accelerator Laboratory (PAL), Pohang, Korea. The raw data were processed and scaled using the program HKL2000. The structure was solved by direct methods, and the refinements carried out with full-matrix least-squares on *F*2 with the appropriate software implemented in the SHELXTL program package.

Cancer cell growth inhibition assay (MTT assay)

The cells were grown at 37 ^{o}C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) 1% penicillin streptomycin. Cell suspensions of different cells were seeded into 96-well plates at a concentration of 5×10^4 cells per well (90 μL per well and 10 μL sample). MTT was first prepared as a stock solution of 5mg/ml in phosphate buffer (PBS, pH 7.2) and was filtered. 10 μL of MTT solution was added to each well. After incubation for 4 h at 37 ^{o}C and 5% CO_2, 100 μL of DMSO (Dimethylsulfoxide) was added to each well. After the 96 well plates was read by an enzyme-linked immunosorbent assay (ELISA) reader at 570 nm for absorbance density values to determine the cell viability and

the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC50 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.

Apoptosis assay

Flow cytometry assay was carried out for the cell-cycle measurement. At the end of sample treatment, cells were trypsinized, washed twice with 1×PBS, and pelleted by centrifugation. Pellet was resuspended with 70% ethanol for 1 h at 4°C. Then cell were incubated with the DNA-binding dry propidiumiodide (PI) solution [0.1% sodium citrate (w/v), 0.1% Triton ×-100 (v/v), and 100μg/ml PI in deionized water] for 30 min at room temperature. Finally, cells were analyzed using FACS caliber flow cytometer (BD, San Jose, CA).

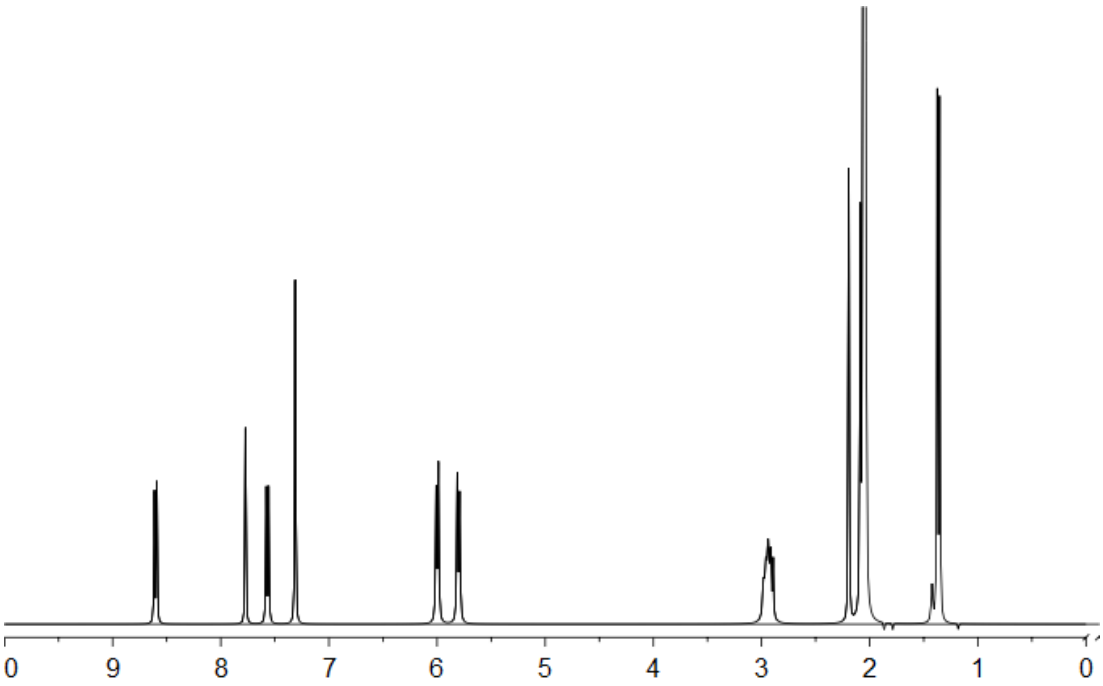
Synthesis of Nano-Prismatic Cage 8: A CH_2Cl_2 solution (0.5 mL) of tripodal donor **5** (1.50mg, 0.004 mmol) was added dropwise to a CH_3OH solution (0.5 mL) of ruthenium triflate acceptor **3** (5.15 mg, 0.006 mmol). The mixture was then stirred for 4 h at room temperature. Upon addition of diethyl ether, a sea-green crystalline powder was afforded. Yield 91 %. ^{1}H NMR (300 MHz, CD_3COCD_3): δ = 8.61 (d, 3*J*H,H = 6.6 Hz, 12 H, Hα), 7.77 (s, 6H ; Hbz), 7.57 (d, 3*J*H,H = 6.6 Hz, 12 H, Hβ), 7.31 (s, 12 H, Hq), 6.00 (d, 3*J*H,H = 6.0 Hz, 12 H, Hcym), 5.81(d, 3*J*H,H = 6.0 Hz, 12 H, Hcym), 2.96 [sept, 3*J*H,H = 6.9 Hz, 6 H, C*H*$(CH_3)_2$], 2.19 (s, 18 H, CH_3), 1.33 [d, 3*J*H,H = 6.9Hz, 36 H,CH(C*H*$_3)_2$] ppm. ^{13}C{^{1}H} NMR (75 MHz, CD_3COCD_3): δ = 171.8 (*C*O), 170.7 (*C*Bz), 154.3 (*C*Hα), 145.2 (*C*Bz), 138.4 (*C*Hq), 125.0(*C*Hβ), 112.5 (*C*q), 104.9 (*C*cym), 100.5 (*C*cym), 85.2 (*C*Hcym), 84.2(*C*Hcym), 31.5 [*C*H$(CH_3)_2$], 23.1 [CH(*C*H$_3)_2$], 22.3

[CH(CH$_3$)$_2$], 17.3(CH$_3$) ppm. MS (ESI) for **8** ($C_{150}H_{126}F_{18}N_6O_{30}Ru_6S_6$): 1667.2 $[M - 2OTf]^{2+}$; 1062.2 $[M - 3OTf]^{3+}$.

Synthesis of Nano-Prismatic Cage 9: A CH_2Cl_2 solution (0.5 mL) of tripodal donor **5** (2.63 mg, 0.007 mmol) was added dropwise to a CH_3OH solution (0.5 mL) of the acceptor **4** (9.37 mg, 0.010 mmol). The mixture was then stirred for 4 h at room temperature. Upon the addition of diethyl ether, a green crystalline solid was formed and collected. Yield 87 %. ^{1}H NMR (300 MHz, CD_3COCD_3): δ = 8.85 (br., 12 H, Hbq), 8.77 (d, 3JH,H = 6.6 Hz, 12 H, Hα), 7.99 (br., 12 H, Hcq), 7.97 (s, 6H ; Hbz), 7.44 (d, 3JH,H = 6.6 Hz, 12 H, Hβ), 6.20 (d, 3JH,H = 6.3Hz, 12 H, Hcym), 5.98 (d, 12 H, Hcym), 3.08 [sept, 3JH,H = 6.9 Hz, 6 H, CH(CH_3)$_2$], 2.26 (s, 18 H, CH_3),1.37 [d, 3JH,H = 6.9Hz, 36 H, CH(CH_3)$_2$] ppm. ^{13}C{1H} NMR (75 MHz, CD_3COCD_3)): δ = 170.5 (Ctpt), 170.0 (CO), 154.1 (CHα), 145.0 (Ctpt),134.6 (Cq), 134.1 (CHcq), 128.2 (CHbq), 124.9 (CHβ), 108.1 (Cq), 104.9 (Ccym), 100.7 (Ccym), 85.1 (CHcym), 83.6 (CHcym), 31.5 [CH(CH_3)$_2$], 22.5 [CH(CH3)2], 17.8 (CH$_3$) ppm. MS (ESI) for **9** ($C_{174}H_{138}F_{18}N_6O_{30}Ru_6S_6$): 1817.6 $[M - 2OTf]^{2+}$, 1162.3 $[M - 3OTf]^{3+}$.

Fig. 1. ^{1}H-NMR spectra of nano-cages. (a) **8** and (b) **9**.

(a)



(b)

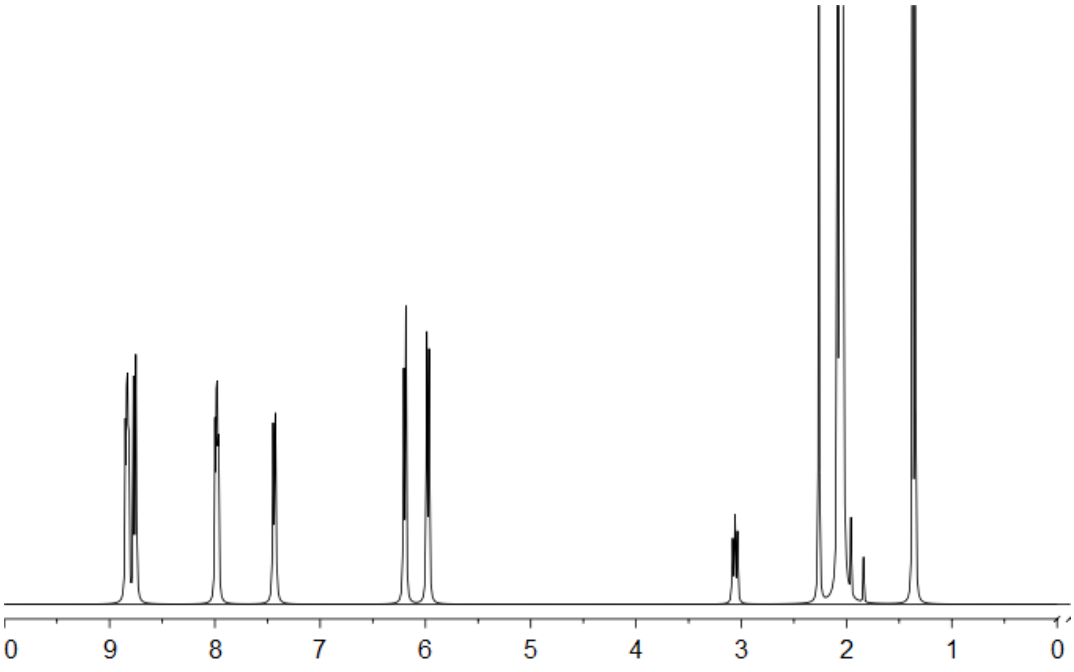
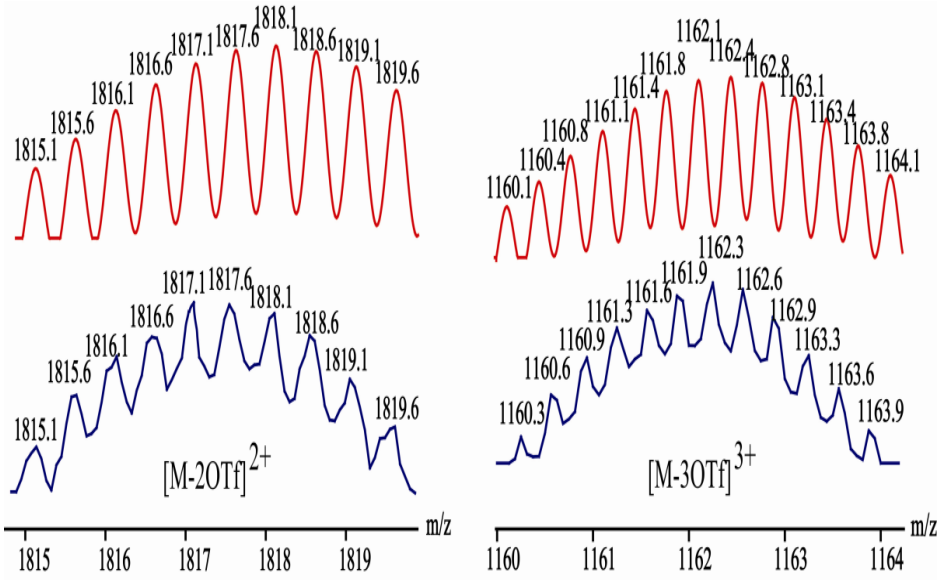


Fig. 2. Calculated (red) and experimental (blue) ESI mass spectra of nano- cages **8** (a) and **9** (b).

(a)



(b)

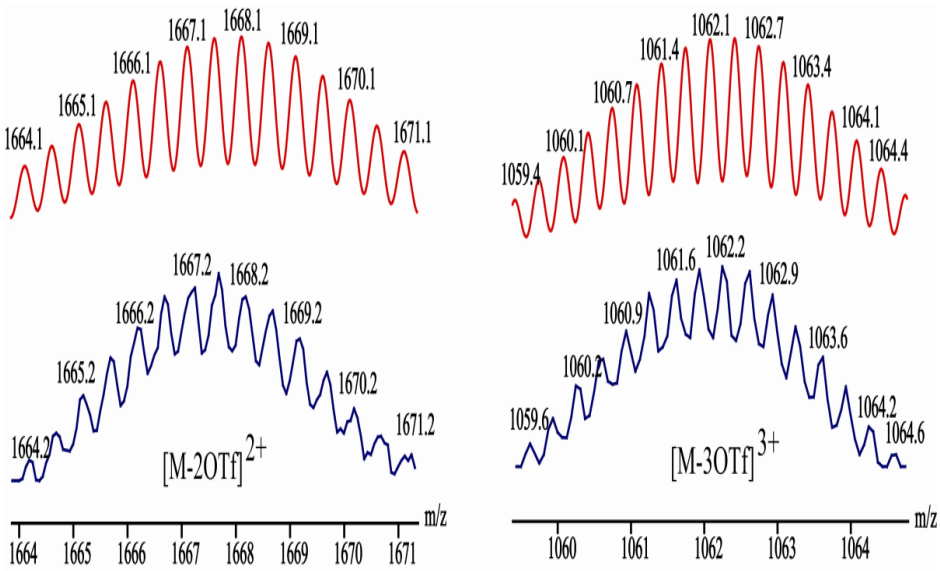


Fig. 3. Numbered crystal diagram for nano-cage **8** Color codes: yellow = Ru, green = C, blue = N, red = O.

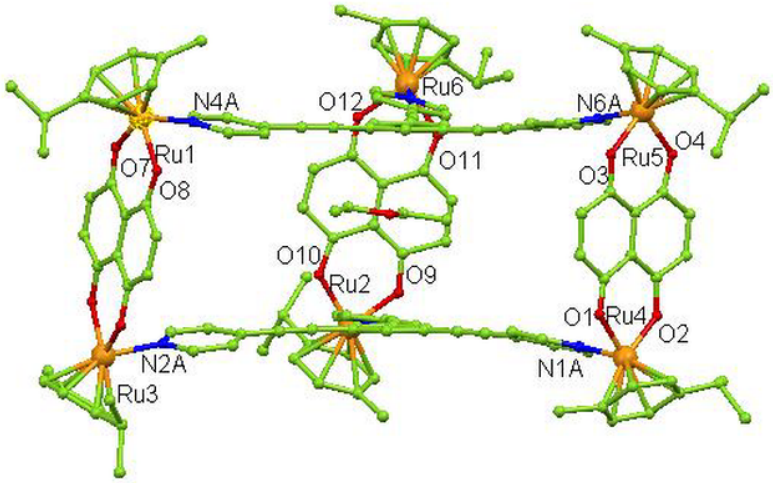


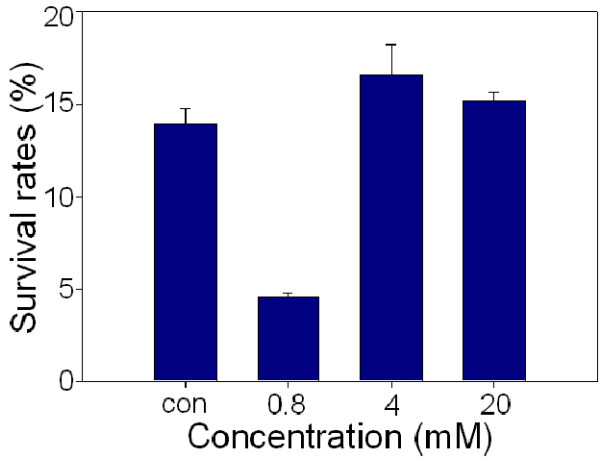
Table 1. Important Bond Lengths (Å) and Angles (º) for the nano-cage **8**.

Ru(1)-O(7)	2.021(7)	Ru(1)-N(4A)	2.102 (9)
Ru(5)-O(3)	2.007(10)	Ru(5)-O(4)	2.020(9)
Ru(5)-N(6A)	2.114(11)		

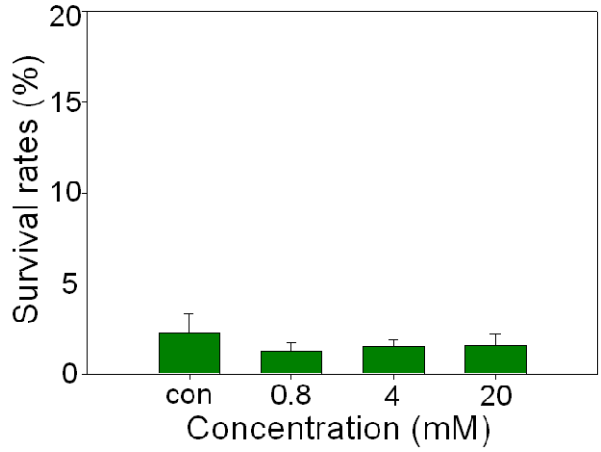
O(7)-Ru(1)-O(8)	87.2(3)	O(7)-Ru(1)-N(4A)	84.9(3)
O(8)-Ru(1)-N(4A)	83.6(3)	O(3)-Ru(5)-O(4)	89.6(5)
O(3)-Ru(5)-N(6A)	83.2(4)	O(4)-Ru(5)-N(6A)	83.8(4)

Fig. 4. The apoptotic effects of ruthenium nano-cage **8** for cell cycles on the A549 cell line. (a) G1 phase cell rates, (b) S phase cell rates and (c) G2/M phase cell rates.

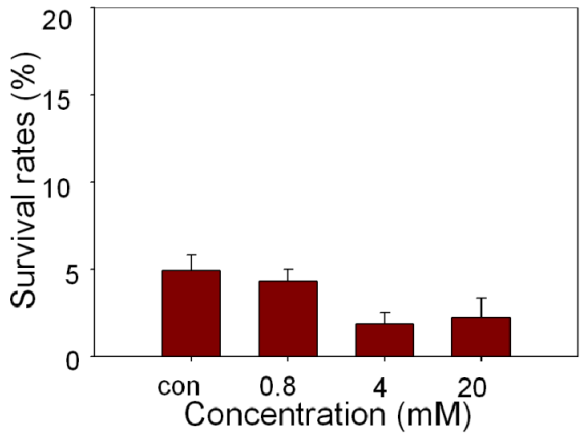
(a)



(b)



(c)



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1. T. Kikuchi, T. Murase, S. Sato and M. Fujita, *Supramolecular. Chem*., 2008, **20**, 81.
2. N. P. E. Barry and Bruno Therrien, *Eur. J. Inorg. Chem.,* 2009, 4695.
3. H.-B. Yang, A. M. Hawkridge, S. D. Huang, N. Das, S. D. Bunge, D. C. Muddiman, and P. J. Stang, *J. Am. Chem. Soc.*, 2007, **129**, 2120.