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A Cyclometallated Platinum Complex as a Selective Optical Switch for Quadruplex DNA

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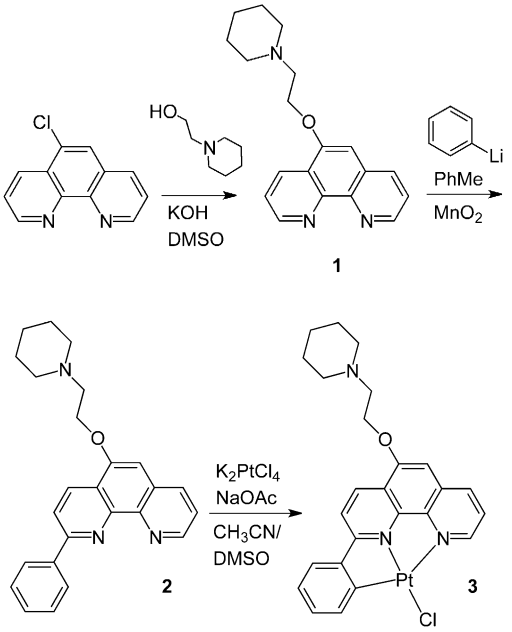
DNA sequences with the potential to form non-canonical structures (such as hairpins, triplexes, quadruplexes and cruciforms) are widespread in genomes. Interestingly, the distribution of such sequences is not random, being selectively enriched in specific genes and, particularly, in transcriptional regions. This strongly suggests that non-canonical DNA structures play important roles in essential biological functions, such as replication, transcription and recombination. Amongst these non-canonical topologies, G-quadruplexes have received increasing attention due to their proposed involvement in gene regulation as well as in telomere maintenance.[1] Therefore, it has been proposed that G-quadruplexes could be interesting targets for the development of anticancer drugs[2] (e.g., by down-regulating oncogene expression,[1b,3] inhibiting telomerase[4]) and extensive research has been carried out towards developing molecules that can selectively target these DNA topologies. Although a wide range of organic and metal–organic molecules have been synthesised towards this aim,[5] far less has been achieved in the development of luminescent probes that can target quadruplexes selectively. Such probes could provide insight into the formation of quadruplexes in vivo as well as being very useful tools for aiding our understanding of DNA biophysical properties. Some recent examples of quadruplex-selective optical probes are Thomas' ruthenium-based optical switches,[6] Luedtke's zinc-phthalocyanine complexes,[7] Che's platinum(II) dipyridophenazine luminescent probes[8] and the quadruplex-templated fluorogenic reactions reported by Ladame.[9] An interesting approach to optical detection of quadruplexes based on assembling platinum(II) complexes has been developed by Yam.[10a] Some organic dyes have also been reported to be optical switches for G-quadruplex DNA.[10b–d]

Herein we report the synthesis of a new phenanthroline-based ligand and its corresponding cyclometallated platinum(II) complex (Scheme 1). We show that this complex interacts selectively with quadruplex DNA and upon binding its emission is switched on. The encapsulation of this complex inside a hexaruthenium cage is also reported as means of transporting the optical probe inside the cell allowing us to carry out optical imaging studies of its cellular targets and localisation.



Scheme 1. Synthesis of phenanthroline-based ligand **2** and the corresponding cyclometallated platinum(II) complex **3**.

As has been extensively discussed elsewhere, complexes containing co-planar aromatic rings, a central metal cation and amine substituents that can be protonated at physiological pH, tend to have good affinities for quadruplex DNA.[5] We have previously shown that platinum(II) and palladium(II) complexes coordinated to monosubstituted phenanthrolines have good affinities towards quadruplexes.[11] On the other hand, Che et al. have previously reported that phenyl-substituted phenathrolines can act as tridentate (CNN) ligands and yield phosphorescent platinum(II) complexes.[12] One of these complexes was previously shown to bind strongly to calf thymus DNA (ct-DNA) but poorly to

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quadruplex DNA.[13] Considering the above, we designed the phenanthroline ligand **2** (Scheme 1) so that it would feature: 1) four co-planar aromatic rings ideally positioned to stack on the G-quartet; 2) an ethyl-piperidine substituent on phenanthroline's backbone to enhance solubility and to hinder the resulting platinum(II) complex from intercalating easily in-between base pairs of duplex DNA (hence providing quadruplex selectivity); 3) a CNN coordination motif to yield platinum(II) complexes with emissive properties. Thus, this ligand and the corresponding platinum(II) complex **3** were prepared as shown in Scheme 1. Ligand **2** was characterised by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analyses (see the Experimental Section). This ligand was then treated with K_2PtCl_4 in the presence of NaOAc for 3 days at 65 °C to yield complex **3** as a yellow crystalline solid. The platinum(II) complex was fully characterised by ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis. The ^{1}H NMR spectrum displayed the expected peaks (ten aromatic and four aliphatic signals with the correct multiplicities and integrals; it should be noted that the fifth expected aliphatic signal from one of the piperidine's CH_2, overlaps with the solvent peak). Importantly, the formation of the cyclometalled Pt–C (aromatic) bond was confirmed by the disappearance of one *ortho* phenyl proton resonance in **2**. A distinctive molecular ion peak was observed in the ESI(+) mass spectrum (*m*/*z* 614 a.m.u., $[M+H]^+$) providing further evidence for complex formation.

Crystals of **3** suitable for an X-ray crystallographic analysis were obtained by slow diffusion of ethyl ether into a dichloromethane solution of the compound. The structure of **3** (Figure 1) shows the phenyl-phenanthroline ligand to have an approximately co-planar conformation, the torsion angle about the C–C bond linking rings **A** and **D** being about 3°.

The platinum has the expected distorted square planar coordination geometry with *cis* angles in the range 80.26(13) to 101.43(12)°. Adjacent centrosymmetrically-related molecules are linked by π–π stacking interactions between phenanthroline rings to form chains along the crystallographic *a* axis direction (Figures S27 and S28 in the Supporting Information). These chains are cross-linked to their C_i-related counterparts by a C–H···π interaction, forming sheets of molecules in the crystallographic *ab* plane.

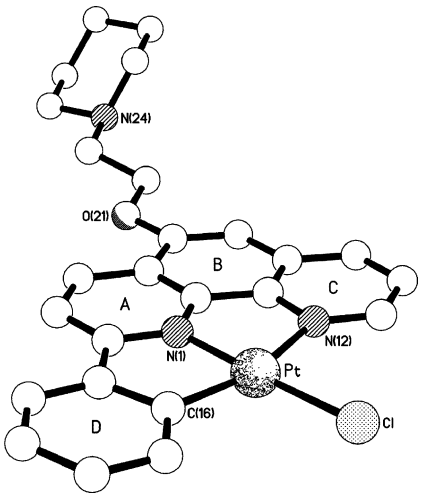


Figure 1. The crystal structure of **3** showing the aryl-ring labelling scheme.

To assess whether **3** also displays π–π stacking interactions in solution, variable temperature and concentration ^{1}H NMR spectroscopic studies were carried out in [D$_6$]DMSO (Figures S3–S6 in the Supporting Information). Upon increasing the temperature of a solution of **3**, the aromatic resonances shifted towards higher field, which—as has been discussed previously for other aromatic systems—is consistent with π–π interactions being disrupted as the temperature is increased.[14] A similar effect was observed when the ^{1}H NMR spectra were recorded at various concentrations: upon decreasing the concentration, a high field shift of the aromatic resonances was observed, consistent with the expected reduction in π–π interactions at lower concentrations. This shows that complex **3** displays π–π interactions in DMSO solution, which is generally a good indication of a complex's potential to interact via π–π stacking with DNA.

Complex **3** was found to be emissive in organic solvents (in CH_2Cl_2, $\lambda_{ex}=430$ nm and $\lambda_{em}=570$, 613 nm; quantum yield = 0.024) but the emission was quenched in water. Due to the well-resolved vibrational structure and by comparison to the emission spectra of previously reported platinum(II) cyclometallated complexes,[12,13] the origin of the emission of **3** centred at 570 nm can be assigned tentatively to the $\pi\rightarrow\pi^*$ intraligand transition. Interestingly, the emission of the complex in aqueous media was restored upon interaction with DNA and, as discussed below, in particular with quadruplex DNA.

The ability of complex **3** to interact with different DNA topologies was first investigated by UV/Vis spectroscopic titrations. Three different quadruplex DNA structures were investigated: our initial studies focussed on the G-rich promoter region of *c-Myc* oncogene. It is well-established that this sequence readily folds into a quadruplex structure, which leads to the down-regulation of *c-Myc*.[1b] In addition, two human ribosomal DNA (rDNA) sequences known to form quadruplexes (6183 NT and 7253 NT)[15] were investigated. As will be discussed later, confocal microscopy studies revealed a high concentration of complex **3** in the nucleoli suggesting that rDNA could be one of its molecular targets. As can be seen in Table 1, complex **3** has high affinity towards quadruplex DNA structures with a 1000-fold selectivity (as measured by UV/Vis titrations) for quadruplexes over a 26-mer duplex DNA (ds26 DNA).

As a result of tight binding, the emission of complex **3** in aqueous solution was shown to "switch-on" in the presence of quadruplex DNA. Therefore, the emission of **3** was recorded in the presence of the four different quadruplexes under study as well as duplex DNA (both ds26- and ct-DNA) and other biomolecules, such as bovine serum albumin (BSA). These studies showed that the enhancement in emission of **3** was much more pronounced in the presence of

Table 1. Binding constants obtained for **3** determined by UV/Vis and emission titrations.[a]

Sequence	K [M^{-1}]	
	UV/Vis	Emission
c-Myc	$6.7(\pm0.7)\times10^7$	$2.1(\pm0.3)\times10^7$
Htelo	$1.3(\pm0.1)\times10^7$	$1.2(\pm0.3)\times10^7$
6183 NT	$4.6(\pm1.5)\times10^7$	$1.4(\pm0.3)\times10^7$
7253 NT	$1.5(\pm0.8)\times10^7$	$8.0(\pm1.5)\times10^6$
ct-DNA	$4.0(\pm1.1)\times10^5$	$1.3(\pm0.8)\times10^5$
ds26 DNA	$5.5(\pm2.4)\times10^4$	n.d.[b]

[a] The values are an average of three independent measurements. [b] The binding constant between complex **3** and ds26 DNA was not determined by emission titration since no measurable change in intensity of the complex's emission could be detected upon interaction with this oligonucleotide (see inset in Figure 2).

c-Myc and 6183 NT quadruplex DNA than in the presence of duplex DNA (see Figure 2 for a graphical representation of the emission response and Table 1 for apparent affinity

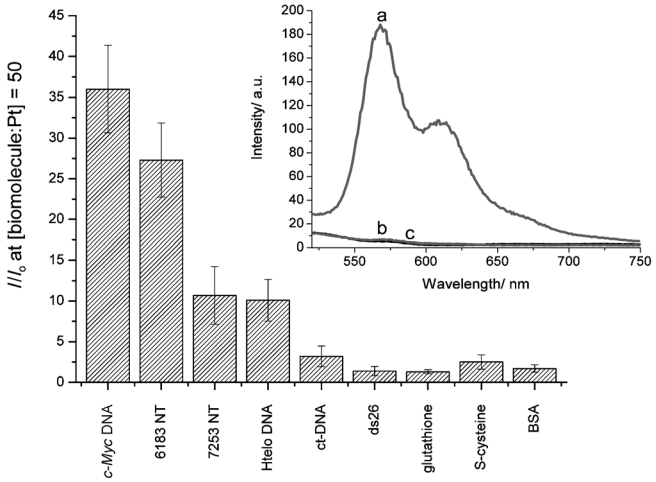
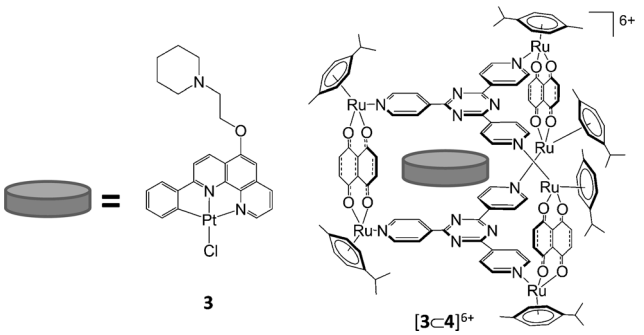


Figure 2. Bar chart depicting the enhancement in the emission of complex **3** (in Tris-KCl buffer) upon addition of 50 equiv of a range of biomolecules. Optical selectivity for *c-Myc* and 6183 NT DNA over duplex DNA is shown. The inset shows the emission spectra of complex **3**+*c-Myc* (a), **3**+ds26 DNA (b) and **3** without DNA (c).

constants determined by emission spectroscopy). Indeed, in the case of ds26 DNA no enhancement of the emission was observed (Figure 2, inset). This highlights the great potential of this complex for the selective detection of quadruplex structures over duplex DNA.

Considering the ability of this complex to switch-on its emission selectively in the presence of quadruplexes, it was of interest to determine whether **3** would be taken up by cells, and its localisation was monitored by confocal fluorescence microscopy. For this, it was first necessary to establish the concentration range at which the molecule could be used; thus, its cytotoxicity (using the MTS assay) against the osteosarcoma U2OS cell line was determined, giving an $IC_{50}=93.3(\pm2.4)$ μM; therefore we chose to employ a concentration of 10–50 μM of the complex for subsequent studies. Complex **3** was incubated at concentrations below 50 μM for 24 h with U2OS cells and the sample was studied by confocal fluorescence microscopy. Unfortunately, under these conditions, confocal fluorescence microscopy showed that the compound was not taken up by U2OS cells preventing us from establishing cellular targets and localisation (Figure S23 in the Supporting Information). To overcome this problem, we decided to explore whether a molecular carrier could be used to aid cellular uptake of complex **3** and consequently enable us to carry out further studies. To this aim, the hexanuclear metalla-prism $[Ru_6(\eta^6\text{-}p\text{-cymene})_6(tpt)_2(donq)_3](CF_3SO_3)_6$ ($[\mathbf{4}](CF_3SO_3)_6$) was investigated (Scheme 2). This supramolecular assembly has been previ-



Scheme 2. Schematic representation of host–guest complex $[\mathbf{3}\subset\mathbf{4}](CF_3SO_3)_6$. The emission of platinum complex **3** was seen to decrease upon encapsulation by host **4**.

ously shown to be an excellent host for guest molecules with planar aromatic substituent.[16] More interestingly, the resulting host–guest assemblies are able to cross the cell membrane and release their cargo after internalisation. Thus, the synthesis of $[\mathbf{3}\subset\mathbf{4}]^{6+}$ was carried out by mixing the hexanuclear cage **4** with the platinum(II) complex **3** for 6 h in dichloromethane. From this reaction a dark green solid was isolated, which was characterised as the novel host–guest complex $[\mathbf{3}\subset\mathbf{4}](CF_3SO_3)_6$ by DOSY NMR spectroscopic experiments as well as by ESI(+)-MS (see the Supporting Information for details of spectroscopic data). The association constant of this assembly was determined by the UV/Vis dilution method[17] to be $3.1\times10^4\,M^{-1}$ (CH_2Cl_2 at 25 °C), which is consistent with analogous host–guest systems reported previously.[16b,18] The emission of complex **3** is almost completely quenched (quantum yield = 0.001) when encapsulated, consistent with previous observations of encapsulation of planar compounds inside cage **4**. Therefore, it was not expected to detect any signal from the caged compound in fluorescence microscopy experiments in cells. The complex–cage assembly $[\mathbf{3}\subset\mathbf{4}](CF_3SO_3)_6$ was then incubated with U2OS cells by using the same procedure as that employed for the free platinum complex **3** (except that for the caged complex a lower concentration of 2 μM was used). After 24 h, confocal fluorescence microscopy studies were carried out. These demonstrated that the host–guest system penetrates the cell, releases the guest molecule, and that a high

proportion of complex **3** accumulates in the nucleus showing specific staining of the nucleoli (Figure 3). Interestingly, co-staining with DAPI (a known duplex DNA binder) showed that the complex does not co-localise with this dye (Figure 3 and Figure S24 in the Supporting Information). This suggests that, rather than duplex DNA, complex **3** interacts with alternative DNA topologies. Previous investigations have reported similar observations where G-quadruplex DNA-selective dyes do not co-localise with DAPI in cellular studies.[10b,19]

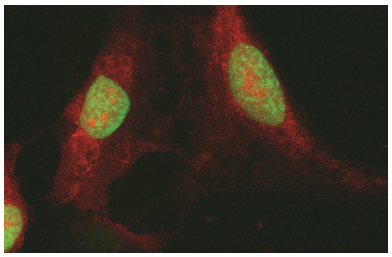


Figure 3. Two-colour fluorescence image of U20S cells incubated with DAPI (0.5 μM) for 30 min ($\lambda_{ex}=405$ nm, $\lambda_{em}=420$–520 nm; shown in green) and 2 μM caged-complex [**3**⊂**4**]$(CF_3SO_3)_6$ for 24 h ($\lambda_{ex}=488$ nm, $\lambda_{em}=550$–700 nm; shown in red). Overlapping regions should appear yellow. The image shows the lack of co-localisation of the platinum(II) probe and DAPI fluorescence.

We also recorded fluorescence spectra of the platinum complex from different regions of the cell (Figure S25 in the Supporting Information). These measurements established that a significant shift in fluorescent maximum (from 570 to 630 nm) is observed in cells as compared to in vitro solution studies. The spectral shape is also markedly different, showing the lack of vibrational structure, which might be characteristic of the emission from the lowest charge transfer excited state, for example, LLCT. Considering that Cl-ligand is relatively labile, we tested a range of species found in the cellular environment that could potentially displace Cl and coordinate to the platinum complex. More specifically, we investigated the emission of complexes resulting from the incubation of **3** with glutathione, cysteine, guanosine and OH (Figure S26 in the Supporting Information). As expected, the emission of the resulting platinum(II) complexes was highly sensitive to substitution of chloride by other ligands. Considerable shifts in the emission maxima were observed. In the case of guanosine, a shift towards the blue ($\lambda_{em}=545$ nm) took place, whereas a red-shift ($\lambda_{em}=740$ nm) was observed in the presence of the S-donor species, cysteine and glutathione. Interestingly, in the presence of aqueous NaOH solution, the emission of the platinum(II) complex shifted to 620 nm (see the emission from the microscopy studies at $\lambda_{em}=630$ nm). These results suggest that the chloride coordinated to platinum in complex **3** is exchanged in the cell by a ligand that shifts the emission towards the red. Also, the shifts observed in the presence of guanosine indicate that complex **3** does not coordinate directly to DNA since the emission recorded during the microscopy studies is not consistent with the shifts seen upon addition of guanosine to complex **3**. It remains to be seen how the quadruplex affinity changes for the OH-substituted platinum complex, compared to its parent Cl analogue; however, we expect a similar level of interaction with the quadruplex due to the minimal structural perturbation to the aromatic core of the complex.

In summary, herein we have presented a new cyclometallated platinum(II) complex the emission of which is switched-on in the presence of DNA. The complex interacts with a 1000-fold selectivity with quadruplex DNA over duplex DNA. More interestingly, its emission is switched-on (ca. 35-fold) in the presence of *c-Myc* and 6183 NT quadruplex DNA, but no significant change in intensity was observed upon addition of the same amounts of duplex ds26 DNA (and only a slight change upon addition of ct-DNA). Upon encapsulation of this complex inside a hexaruthenium cage, we have been able to deliver **3** inside living cells, allowing us to carry out confocal fluorescence microscopy studies. These studies have shown that the compound is released from the cage once inside the cell and a considerable amount of it penetrates the nucleus and localises in the nucleoli. Interestingly, complex **3** does not co-localise with DAPI (a duplex DNA binder) suggesting that it does not target duplex DNA. Considering the cellular localisation of the probe (in the nucleoli) it is possible that alternative DNA topologies, such as quadruplexes are being targeted. However, further studies will be required to confirm this.

Experimental Section

Synthesis of 5-(1-ethyloxy)-piperidine-1,10-phenanthroline (1): 5-Chloro-1,10-phenanthroline (0.200 g, 0.94 mmol) and 1-(2-hydroxyethyl)piperidine (146 mg, 0.94 mmol) were slowly added to a stirred suspension of powered KOH (264 mg, 5.0 mmol) in DMSO (5 mL). The solution was stirred under nitrogen at 60 °C for 4 h. The reaction mixture was extracted with DCM (50 mL × 3), washed thoroughly with water (30 mL × 3) and dried over sodium sulfate. The solvent was removed under reduced pressure to yield the product as a brown solid (212 mg, 73 %). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta_H=9.23$ (dd, 1H, $^3J_{HH}=6.0$, 2.0 Hz, phen 2-H), 9.05 (dd, 1H, $^3J_{HH}=6.0$, 2.0 Hz, phen' 2-H), 8.71 (dd, 1H, $^3J_{HH}=8.0$, 2.0 Hz, phen 4-H), 8.14 (dd, 1H, $^3J_{HH}=8.0$, 2.0 Hz, phen' 4-H), 7.68 (dd, 1H, $^3J_{HH}=8.0$, 4.0 Hz, phen 3-H), 7.59 (dd, 1H, $^3J_{HH}=8.0$, 4.0 Hz, phen' 3-H), 7.00 (s, 1H, phen 5-H), 4.44 (t, 2H, $^3J_{HH}=6.0$ Hz, ethyl 1-H), 3.03 (t, 2H, $^3J_{HH}=6.0$ Hz, ethyl 2-H), 2.65 (m, 4H, pip 1-H), 1.67 (m, 4H, pip 2-H), 1.51 ppm (m, 2H, pip 3-H); ^{13}C NMR (400 MHz, [D$_6$]DMSO): $\delta_C=$ 152.1, 150.6, 147.9, 134.6, 130.9, 129.2, 123.7, 123.2, 122.6, 101.7, 66.9, 57.8, 55.2, 26.0, 24.1 ppm; ESI-MS calcd for $C_{19}H_{21}N_3O$ $[M]^+$: 307.2 a.m.u.; found $[M+H]^+$: 308.0 a.m.u.; elemental analysis calcd for $C_{19}H_{21}N_3O{\cdot}1.45\,H_2O$: C 68.42, H 7.22, N 12.60; found: C 68.86, H 6.76, N 12.58.

Synthesis of 2-phenyl-5-(1-ethyloxy)-piperidine-1,10-phenanthroline (2): 2-Phenyl-5-(1-ethyloxy)-piperidine-1,10-phenanthroline (0.200 g, 0.65 mmol) was dissolved in dry toluene (100 mL) under N_2 atmosphere and the solution was cooled to 0 °C. Phenyllithium (2.0 M solution in dibutyl ether, 0.32 mL, 0.65 mmol) was then added dropwise in a manner as described by Jakobsen.[20] The resultant deep-brown solution was stirred under nitrogen atmosphere at 0 °C for 3 h. After this period MeOH (10 mL) was added to quench the reaction and the products were re-oxidised by incubating the solution with MnO_2 (3 g) for 24 h. The solution was then filtered through Celite and the solvent was removed to give a

crude product as a white/yellow oil. Purification was performed by precipitation/recrystallisation from cold CH_3CN to give a pure product as a white solid (0.19 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 9.09 (d, 1H, $^3J_{HH}$ = 4.0 Hz, phen 2-H), 8.76 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phen 4-H), 8.37 (d, 2H, $^3J_{HH}$ = 4.0, 8.0 Hz, phen 3-H), 8.36 (d, 2H, $^3J_{HH}$ = 4.0, 8.0 Hz, phen 7-H), 8.14 (m, 2H, phen 8-H, phenyl 5-H), 8.57 (m, 3H, phenyl 3-H 4-H, 5-H), 7.50 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phenyl 2-H), 7.69 (s, 1H, $^3J_{HH}$ = 8.0 Hz, phen 6-H), 4.45 (brs, ethyl 1-H), 3.04 (brs, 2H, ethyl 2-H), 2.66 (brs, 4H, piperidine 1-H), 1.68 (brs, 1H, piperidine N$^+$-H), 1.60 (brs, 4H, piperidine 2-H), 1.52 ppm (brs, 2H, piperidine 3-H); ^{13}C NMR (400 MHz, $CDCl_3$): δ_C = 157.8, 152.2, 148.0, 146.6, 143.1, 139.6, 134.8, 131.8, 129.7, 129.4, 128.8, 128.0, 123.1, 122.4, 120.1, 101.6, 66.8, 57.8, 55.2, 26.0, 24.1 ppm; ESI-MS calcd for $C_{25}H_{25}N_3O$ $[M]^+$: 383.2 a.m.u.; found $[M+H]^+$: 384.0 a.m.u.; IR: $\tilde{\nu}$ = 2935 (w), 1616 (m), 1493 (w), 1395 (m), 1307 (m), 1159 (m), 1124 (m), 1081 (m), 990 (m), 832 (m), 839 (m), 742 (s), 692 cm^{-1} (s); elemental analysis calcd for: $C_{25}H_{25}N_3O$: C 78.30, H 6.57, N 10.96; found: C 78.18, H 6.60, N 10.80.

Synthesis of chloro-(2-phenyl-5-(1-ethyloxy)-piperidine-1,10-phenanthroline) platinum(II) (3): Compound **2** (0.100 g, 0.26 mmol) and sodium acetate (0.021 g, 0.26 mmol) were stirred in acetonitrile (15 mL) for 10 min. K_2PtCl_4 (0.108 g, 0.26 mmol) and DMSO (0.5 mL) were then added and the reaction mixture was stirred at 65 °C for 3 days while a yellow precipitate formed. The volume of the solution was reduced to about 5 mL and pentane (20 mL) was added. The resulting precipitate was filtered off, washed with water (30 mL), pentane (15 mL), diethyl ether (15 mL) and dried in vacuo. The solid was re-dissolved in DCM, filtered off and the volume of the filtrate was reduced to about 5 mL. Addition of pentane afforded a pure compound as a yellow crystalline material (112 mg, 70%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ_H = 8.84 (d, 1H, $^3J_{HH}$ = 4.0 Hz, phen 2-H), 8.65 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phen 4-H), 8.61 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phen 7-H), 8.20 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phen 8-H), 8.03 (dd, 1H, $^3J_{HH}$ = 4.0, 4.0 Hz, phen 3-H), 7.73 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phenyl 2-H), 7.56 (d, 1H, $^3J_{HH}$ = 8.0 Hz, phenyl 5-H), 7.51 (s, 1H, phen 6-H), 7.22 (d, 1H, $^3J_{HH}$ = 8.0, 8.0 Hz, phenyl 4-H), 7.14 (d, 1H, $^3J_{HH}$ = 8.0, 8.0 Hz, phenyl 3-H), 4.90 (t, 2H, $^3J_{HH}$ = 4.0 Hz, ethyl 1-H), 2.90 (t, 2H, $^3J_{HH}$ = 4.0 Hz, ethyl 2-H), 1.55 (m, 4H, piperidine 2-H), 1.43 ppm (m, 2H, piperidine 3-H); ESI-MS calcd for $C_{25}H_{24}ClN_3OPt$ $[M]^+$: 612.1 a.m.u.; found $[M+H]^+$: 614.0 a.m.u.; IR: $\tilde{\nu}$ = 2939 (w), 1623 (m), 1453 (m), 1391 (m), 1302 (m), 1257 (m), 1132 (m), 993 (m), 835 (m), 768 (m), 733 (s), 673 cm^{-1} (m); UV/Vis (CH_2Cl_2): λ (ε) = 261 (14650), 321 (8300), 390 (3700), 438 nm (sh) (2158 dm^{3}mol^{-1}cm^{-1}); UV/Vis (aqueous Tris-HCl/KCl buffer): λ (ε) = 266 (15920), 325 (8383), 403 nm (sh) (3825 dm^{3}mol^{-1}cm^{-1}); elemental analysis calcd for $C_{25}H_{24}ClN_3OPt{\cdot}0.5H_2O$: C 48.27, H 4.05, N 6.76; found: C 48.16, H 3.91, N 6.49.

Synthesis of cage–probe assembly [3⊂4](CF$_3$SO$_3$)$_6$: The hexanuclear metalla-prism $[Ru_6(\eta^6\text{-p-cymene})_6(tpt)_2(donq)_3](CF_3SO_3)_6$ ([**4**](CF$_3$SO$_3$)$_6$) [60 mg, 0.017 mmol] and platinum complex **3** (10.5 mg, 0.017 mmol) in dichloromethane (40 mL) were stirred for 6 h at room temperature. The reaction mixture was filtered and concentrated in vacuo. The dark residue was dissolved in dichloromethane (1 mL) and diethyl ether (40 mL) was added to precipitate a dark-green solid which was dried in vacuo (69 mg, 98%). ^{1}H NMR (400 MHz, CD_3CN, 298 K): δ = 8.37 (br, H$_\alpha$), 7.92 (br, H$_\beta$), 7.61–7.31 (overlapped, H$_\beta$+H$_q$), 7.20 (br, 1H, H$_{ar-g}$), 6.91 (br, 1H, H$_{ar-g}$), 6.39 (br, 1H, H$_{ar-g}$), 6.26 (br, 2H, H$_{ar-g}$), 5.86 (br, 1H, H$_{ar-g}$), 5.78 (br, 1H, H$_{ar-g}$), 5.69 (d, ^3J = 5.7 Hz, 12H, H$_{cym}$), 5.47 (d, ^3J = 5.7 Hz, 12H, H$_{cym}$), 5.25 (br, 1H, H$_{ar-g}$), 4.83 (br, 1H, H$_{ar-g}$), 4.66 (br, 1H, H$_{ar-g}$), 4.44 (br, 2H, CH_2O), 2.98 (br, 2H, NCH_2CH_2O), 2.82 (sept, ^3J = 6.7 Hz, 6H, $CH(CH_3)_2$+4H, CH_2N), 2.04 (s, 18H, CH_3), 1.84 (br, 4H, CH_2CH_2N), 1.70 (br, 2H, $CH_2(CH_2)_2N$), 1.30 ppm (d, 36H, ^3J = 6.7 Hz, $CH(CH_3)_2$); ^{13}C{^{1}H} NMR (101 MHz, CD_3CN, 298 K): δ = 171.9 (CO), 170.1 (C$_{tpt}$) 153.6, 153.5, 153.9 (CH$_\alpha$), 144.4 (CH$_{ar-g}$), 138.9, 138.7, 138.4 (CH$_q$), 135.0 (CH$_{ar-g}$), 133.2 (CH$_{ar-g}$), 132.3 (CH$_{ar-g}$), 130.7 (CH$_{ar-g}$), 125.6 (CH$_{ar-g}$), 125.0 (CH$_{ar-g}$), 125.0 (CH$_\beta$), 123.9 (CH$_\beta$), 115.1 (CH$_{ar-g}$), 103.0 (CH$_{ar-g}$), 104.8 (C$_{ar-cym}$), 100.8 (C$_{ar-cym}$), 85.0 (CH$_{ar-cym}$), 84.1 (CH$_{ar-cym}$), 68.8 (CH_2O), 58.5 (NCH_2CH_2O), 56.3 (CH_2N), 31.5 $CH(CH_3)_2$, 27.4 (CH_2CH_2N), 25.3 ($CH_2(CH_2)_2N$), 22.1 ($CH(CH_3)_2$), 17.2 ppm (CH_3). FTMS+ pNSI (m/z): 1270.43 [**3**+**4**+4CF$_3$SO$_3$+H]$^{3+}$, 915.83 [**3**+**4**+3CF$_3$SO$_3$+H]$^{4+}$; UV/Vis (MeOH): λ (ε) = 708 (8800), 658 (8500), 445 (35200), 324 (63700), 228 nm (195000 dm^{3}mol^{-1}cm^{-1}); IR (KBr): $\tilde{\nu}$ = 3066 (w, CH_{ar}), 2965 (s, CH_2), 2926 (s, CH_2), 1622 (s, CO_{donq}), 1574 (s, CC_{tpt}), 1536 (s, CN_{tpt}), 1275 (s, CN), 1259 (s, CF_3), 1158 cm^{-1} (s, CO).

Crystal data for 3: $C_{25}H_{24}ClN_3OPt{\cdot}CH_2Cl_2$, M_r = 697.94, triclinic, $P\bar{1}$ (no. 2), a = 7.9519(3), b = 9.2885(3), c = 18.3759(6) Å, α = 78.766(3), β = 87.360(3), γ = 66.949(3)°, V = 1224.28(8) Å^3, Z = 2, ρ_{calcd} = 1.893 g cm^{-3}, $\mu(Cu_{K\alpha})$ = 13.934 mm^{-1}, T = 173 K, orange needles, Oxford Diffraction Xcalibur PX Ultra diffractometer; 4765 independent measured reflections (R_{int} = 0.0298), F^2 refinement,[20] R_1(obs) = 0.0266, wR_2(all) = 0.0653, 4405 independent observed absorption-corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta_{max}$ = 145°], 307 parameters. CCDC-891672 (**3**) contains 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.

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