Enhanced kinetic stability of [Pd$_2$L$_4$]$^{4+}$ cages through ligand substitution†

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There is considerable interest in exploiting metallosupramolecular cages as drug delivery vectors. Recently, we developed a [Pd$_2$L$_4$]$^{4+}$ cage capable of binding two molecules of cisplatin. Unfortunately, this first generation cage was rapidly decomposed by common biologically relevant nucleophiles. In an effort to improve the kinetic stability of these cage architectures here we report the synthesis of two amino substituted tripyridyl 2,6-bis(pyridin-3-ylethynyl)pyridine (tripy) ligands (with amino groups either in the 2-(2A-tripy) or 3-(3A-tripy) positions of the terminal pyridines) and their respective [Pd$_2$(tripy)$_4$]$^{4+}$ cages. These systems have been characterised by $^1$H, $^{13}$C and DOSY NMR spectroscopies, high resolution electrospray mass spectrometry, elemental analysis and, in one case, by X-ray crystallography. It was established, using model palladium(II) N-heterocyclic carbene (NHC) probe complexes, that the amino substituted compounds were stronger donor ligands than the parent system (2A-tripy $>$ 3A-tripy $>$ tripy). Competition experiments with a range of nucleophiles showed that these substitutions lead to more kinetically robust cage architectures, with [Pd$_2$(2A-tripy)$_4$]$^{4+}$ proving the most stable. Biological testing on the three ligands and cages against A549 and MDA-MB-231 cell lines showed that only [Pd$_2$(2A-tripy)$_4$]$^{4+}$ exhibited any appreciable cytotoxicity, with a modest IC$_{50}$ of 36.4 ± 1.9 µM against the MDA-MB-231 cell line. Unfortunately, the increase in kinetic stability of the [Pd$_2$(L$_{tripy}$)$_4$]$^{4+}$ cages was accompanied by loss of cisplatin-binding ability.

Introduction

Interest in self-assembled coordination complexes of well-defined two- and three-dimensional geometries, or metallosupramolecular architectures, continues to grow due to their potential in a range of applications. The molecular recognition properties of these systems have been used to develop molecular reactions flasks, catalysts and drug delivery agents. Systems have also been used to sequester reactive species, and environmental pollutants. Additionally, the biological, photophysical, electronic and redox properties of these metallosupramolecular architectures have been studied.

As part of our interest in the biological properties of metallosupramolecular architectures we have previously reported the synthesis of a tripyridyl (tripy) [Pd$_2$L$_3$]$^{3+}$ cage capable of binding cisplatin (cis-[Pt(NH$_3$)$_2$Cl$_2$]). We hoped to exploit these [Pd$_2$L$_3$]$^{3+}$ cages as metallosupramolecular drug delivery vectors, unfortunately the cage architecture was rapidly decomposed when exposed to chloride (Cl$^-$), histidine (his)$_d$, histidine (his)$_b$ and cysteine (cys)$_b$ (common nucleophiles in biological systems). In order to use these cisplatin binding [Pd$_2$L$_4$]$^{4+}$ cages as drug delivery agents a method for increasing the kinetic stability of these metallosupramolecular architectures against nucleophiles was required. There are two obvious approaches to enhance the kinetic stability of the cage architectures: (1) assemble the cage using more kinetically inert metal ions or (2) sterically and electronically tune the tripy ligand framework. Exploiting kinetically inert metals for the generation of metallosupramolecular architectures can be difficult due to the formation of kinetically “trapped” intermediate structures that cannot “error-correct” into the desired system. Therefore we chose to generate more electron-donating tripyridyl ligands in an effort to obtain more kinetically robust [Pd$_2$L$_4$]$^{4+}$ cages. Herein we report the synthesis of two new amino substituted tripyridyl ligands (2A-tripy and 3A-tripy) and their respective cages (Scheme 1). The addition of the amino groups to the 2- and 3-positions of the terminal ligating pyridyl units of the ligand framework was expected to increase the donor properties of the ligands and lead to more kineti-
Results and discussion

Synthesis and characterisation

The electronically and sterically tuned tripyridyl ligands (tripy, 2A-tripy, and 3A-tripy, Scheme 1) were synthesised using standard methods through amino substitution in the 2-position circumscribe the ability of the cage to encapsulate cisplatin. Preliminary biological nucleophiles showed that the [Pd2(tripy)4](BF4)4 cage was more stable than the [Pd2(2A-tripy)4](BF4)4 cage (ESI†). The [Pd2(3A-tripy)4](BF4)4 cage displays a very large (Δδ(HNH) = 1.56 ppm) shift relative to the free tripy ligands, consistent with complexation to palladium(II) ions. Interestingly, the proton resonance of the 2-amino group in the [Pd2(2A-tripy)4](BF4)4 cage displays a very large (Δδ(HNH) = 0.81 ppm, ESI†) shift relative to the free ligand. Consistent with this postulate, the shift of the corresponding proton resonance in the [Pd2(3A-tripy)4](BF4)4 cage is much smaller (Δδ(HNH) = 0.19–0.59 ppm) relative to the free tripy ligands, consistent with complexation to palladium(II) ions.

Addition of one of the ligands (tripy, 2A-tripy, and 3A-tripy) to a solution of [Pd(CH3CN)4](BF4)2 in a 2 : 1 ratio resulted in the formation of the desired cages (Scheme 1). While the tripy and 3A-tripy containing cages formed instantaneously at room temperature, the formation of [Pd2(2A-tripy)4](BF4)4 required heating in DMSO solution at 50 °C for six hours to complete the assembly. The cages were characterised using 1H and DOSY NMR spectroscopy, HR-ESMS, and elemental analysis (Experimental section and ESI†). The 1H NMR spectra (Fig. 1 and ESI†) of the [Pd2(Ltripy)4]4+ cages show a single set of peaks. The proton resonances due to the terminal pyridyl units of the cages (Hc–i) are shifted downfield (Δδ = 0.19–0.59 ppm) relative to the free tripy ligands, consistent with coordination to palladium(II) ions. Interestingly, the proton resonance of the 2-amino group in the [Pd2(2A-tripy)4](BF4)4 cage displays a very large shift relative to the free ligand (Fig. 1). Presumably, this large shift is caused by a combination of coordination to the palladium(II) ions and intraligand hydrogen bonding interactions between the amino groups on the adjacent ligands. Consistent with this postulate, the shift of the corresponding proton resonance in the [Pd2(3A-tripy)4](BF4)4 cage is much smaller (Δδ(HNH) = 0.81 ppm, ESI†). The sharp, uncomplicated signals observed in the 1H NMR spectra of the [Pd2(Ltripy)4]4+ systems are similar to what was previously observed for the formation of other [Pd2(L)4]4+ cages and are consistent with the formation of complexes of high symmetry in solution.11,10,6

Diffusion-ordered 1H NMR spectroscopy (DOSY) provided additional strong support for the selective formation of the cages in solution.1H DOSY spectra (d6-DMSO, 298 K) were obtained for ligands (tripy, 2A-tripy, and 3A-tripy) and cages ([Pd2(tripy)4]4+, [Pd2(2A-tripy)4]4+ and [Pd2(3A-tripy)4]4+ (ESI†)). Each of the proton signals in the individual spectra show the same diffusion coefficient (D), indicating that there is only one species present in solution (ESI†). The Dcomplex/Dligand ratios of ~0.50 : 1 are similar to those observed for related literature.
compounds\textsuperscript{11a,d,12} and suggest that the palladium(II) cage species are stable in solution (ESI\textsuperscript{†}).

Mass spectra (HR-ESMS) of the [Pd\textsubscript{2}(L\textsubscript{tripy})\textsubscript{4}]\textsuperscript{4+} cage systems in DMF–CH\textsubscript{3}CN solution obtained under pseudo cold-spray conditions displayed overlapping peaks due to [Pd\textsubscript{2}L\textsubscript{4}]\textsuperscript{4+} and [Pd\textsubscript{2}L\textsubscript{2}]\textsuperscript{2+} ions. Additionally, the spectrum of the 3A-tripy based cage displayed a peak due to the [Pd\textsubscript{2}(3A-tripy)\textsubscript{4}(BF\textsubscript{4})\textsubscript{2}]\textsuperscript{2+} ion.

After considerable effort (>50 crystallisations and data collections) the solid state structure of the [Pd\textsubscript{2}(2A-tripy)\textsubscript{4}][BF\textsubscript{4}]\textsubscript{4} complex was obtained using X-ray crystallography (Fig. 2 and ESI\textsuperscript{†}). Small weakly diffracting X-ray quality crystals were generated by vapour diffusion of diethyl ether into a CH\textsubscript{3}CN solution of the [Pd\textsubscript{2}(2A-tripy)\textsubscript{4}][BF\textsubscript{4}]\textsubscript{4} cage. Although the weak diffraction was, at least in part, due to the presence of multiple disordered solvent molecules and counter anions within the crystal lattice (\textit{vide infra}), the cationic framework of the cage was readily identified (Fig. 2).

The solid state structure of the [Pd\textsubscript{2}(2A-tripy)\textsubscript{4}]\textsuperscript{4+} cage confirms that the coordination of the 2-amino pyridyl units to the palladium(II) ions was monodentate through the pyridyl nitrogens as expected.\textsuperscript{15} Additionally, the 2-amino units of the ligands are engaged in intra-ligand hydrogen bonding interactions (N⋯N 3.78(3) Å, N–H⋯N 2.94 Å, Fig. 2b and ESI\textsuperscript{†}) consistent with \textit{1H} NMR data described above. In contrast to the [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+} cage,\textsuperscript{16} the [Pd\textsubscript{2}(2A-tripy)\textsubscript{4}]\textsuperscript{4+} cation adopts a more twisted structure in which the ligands of the 2A-tripy cages are significantly bent out of planarity and this appears to be caused by hydrogen bonding interactions between the amino groups on the \textit{exo} faces of the architecture. This is quite different to what has been previously observed in the solid state structures of unsubstituted [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+} cations, these cages without the 2-amino groups all were found to adopt a lantern shape, with essentially planar tripy ligands.\textsuperscript{11a,d,12}

The coordinated 2A-tripy ligand distorts in two ways: a swirling of the coordinating pyridine rings relative to the principal rotation axis of the molecule (\(\theta = 34.44°\text{–}34.64°\), compared with \(\theta = 3.47°\text{–}9.42°\) for [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+}) and a twisting of the central pyridine out of the plane through which the ligand coordinates to the two Pd(II) centres (\(\varphi = 35.10°\) compared with \(\varphi = 5.61°\) for [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+}). The cavity dimensions also differ (a Pd⋯Pd distance of 11.530(9)–11.610(9) Å compared with 11.201(1) Å for [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+}, and a core-to-core pyridyl N⋯N distance of 10.711(9)–10.732(9) Å compared with 11.07(1)–11.26(1) Å for [Pd\textsubscript{2}(tripy)\textsubscript{4}]\textsuperscript{4+}, Table 1).

Interestingly, the central cavity of the [Pd\textsubscript{2}(2A-tripy)\textsubscript{4}]\textsuperscript{4+} cage is filled in the solid state. The \textit{exo}-methylene alcohol (CH\textsubscript{2}OH) substituents from the four neighbouring cages in the crystal lattice penetrate into the cavity of each cage and form a hydrogen bonding interaction (O⋯–N 2.74(3) Å, O⋯H⋯N 1.91 Å, Fig. 3 and ESI\textsuperscript{†}) with the endo-pyridyl unit. These interactions generate 2D supramolecular sheets of cages through the solid state structure (Fig. 3 and ESI\textsuperscript{†}).

\textbf{Competition experiments, cisplatin binding and cytotoxicity studies}

The relative pK\textsubscript{a} values of 2-aminopyridine (6.82), 3-aminopyridine (6.04), and pyridine (5.23) indicate that 2-aminopyridine is the most basic ligand.\textsuperscript{17} Evidence that 2-aminopyridine was also the strongest nucleophile was obtained using the palladium(II)-N-heterocyclic carbene (NHC) probe system developed by Huynh and coworkers (Table 2 and ESI\textsuperscript{†}).\textsuperscript{18} Consistent with the pK\textsubscript{a} values, the probe complexes indicated that 2-aminopyridine (161.2 ppm) is a stronger donor than 3-aminopyridine (159.8 ppm) which is a stronger donor than pyridine (159.3 ppm). The chemical shift observed for the 2-aminopyridine ligand is very similar to that previously reported for N-methylimidazole (161.1 ppm)\textsuperscript{18b} suggesting that the donor strength of these ligands are similar. To allow direct comparison to the literature pK\textsubscript{a} values (Table 2) and for synthetic convenience\textsuperscript{11a} we have examined the probe complexes of the simpler pyridine rather than the tripyridyl ligands. However, these pyridine model systems can serve as proxies for their respective tripyridyl ligands (2A-tripy, 3A-tripy, tripy) and provide indirect experimental evidence for the donor properties of the tripy ligands because the steric and electronic changes on going from the pyridine to tripy ligands are the
same across the series. Thus the model complexes provide a qualitative ranking of the substituents’ effects present in the tripy ligands and strongly suggest that the donor properties of the tripyridyl ligands follow the order 2A-tripy > 3A-tripy > tripy.

Table 1  Selected crystallographic distances and angles from [Pd2(2A-tripy)4]4+ and [Pd2(tripy)4]4+.15

|                | [Pd2(2A-tripy)4]4+ | [Pd2(tripy)4]4+ |
|----------------|-------------------|-----------------|
| Θ (°)          | 34.44°–34.64°     | 34.7°–9.42°     |
| Φ (°)          | 35.10°            | 5.61°           |
| d(Pd(a)…Pd(a)) (Å) | 11.53(9)–11.610(9) | 11.201(1) Å    |
| d(Ncore…Ncore) (Å) | 10.71(9)–10.73(9) | 10.07(1)–11.26(1) Å |

* Θ: the swivelling of the coordinating pyridine rings relative to the principal rotation axis of the molecule.  
* Φ: twisting of the central tripyridine out of the plane through which the ligand coordinates to the two Pd(a) centres.

The kinetic stability of the [Pd2(Ltripy)4]4+ architectures in the presence of common biological nucleophiles (Cl−, his and cys) was determined using 1H NMR competition experiments (Table 2 and ESI†). Time-course 1H NMR competition experiments were carried out in 3 : 2 d6-DMF/D2O where 3 mM solutions of each cage were treated with 8 equivalents of tetramethylammonium chloride or 4 equivalents of his or cys. Under these conditions the unsubstituted [Pd2(tripy)4]4+ cage was rapidly decomposed by all the nucleophiles. The half-life for the decomposition of the [Pd2(tripy)4]4+ complex with his was 18 minutes. Despite the [Pd2(3A-tripy)4]4+ architecture containing the slightly more electron rich 3A-tripy ligand the cage was still quickly decomposed by each of the nucleophiles. However, the t1/2 were subtly increased against all the nucleophiles (for his t1/2 = 25 min) suggesting that the enhanced ligand donor properties of the 3A-tripy ligand does lead to increased cage stability relative to the unsubstituted system.

The [Pd2(2A-tripy)4]4+ cage displayed markedly higher stability against all the nucleophiles studied. The half-lives for the 2A-tripy cage decomposition against each nucleophile were all over 2 h, whereas the corresponding t1/2 for the other cages were all less than 30 min. 2A-tripy is only a modestly stronger donor ligand than the 3A-tripy, and thus the observed large difference in stability is presumably not predominantly due to the increase donor ability of the ligand. A more important element is likely to be the presence of the 2-amino groups on the exo-faces of the [Pd2(2A-tripy)4]4+ cage which sterically protect the palladium(II) ions from the incoming nucleophiles. Additionally, as observed in the X-ray structure (Fig. 3), the hydrogen bonding interactions between the eight amino groups of the four 2A-tripy ligands may further enhance the stability of the [Pd2(2A-tripy)4]4+ cage relative to the other tripy architectures. However, against the stronger nucleophiles (Cl− and cys) the half-lives for the decomposition of the [Pd2(2A-tripy)4]4+ are less than 3 h suggesting that these systems would need further tuning in order to be useful in a biological setting.

Cisplatin binding

We1,13,18,19 and others16 have previously shown that other similar [Pd2(tripy)4]4+ cages can encapsulate cisplatin through hydrogen bonding interactions in CH3CN and DMF solvents.20
1H NMR spectroscopy and recently reported crystallographic evidence\textsuperscript{16d} confirms that despite the presence of the potential hydrogen bond donor CH$_3$OH units of the exo surface of the [Pd$_2$(tripy)$_4$]$^{4+}$ cage the system retains the ability to bind cisplatin. Addition of an excess of cisplatin to a $d_7$-DMF solution of [Pd$_2$(tripy)$_4$]$^{4+}$ resulted in a large downfield shift ($\Delta \delta = 0.24$ ppm) of the internally directed cage proton H$_c$ (Fig. 4a and b) indicative of cisplatin binding within the cage cavity. The solid state structure\textsuperscript{16b} of the [(clisatin)$_2$Pd$_2$(tripy)$_4$]$^{4+}$ host–guest adduct has recently been reported by Casini, Kuhn and co-workers and is very similar to what we have previously observed in related systems.\textsuperscript{11d,16b} Consistent with the 1H NMR evidence, the [Pd$_2$(tripy)$_4$]$^{4+}$ cation binds two molecules of cisplatin within the cavity of the cage.\textsuperscript{16} The guest molecules are rotated 180° with respect to each other; hydrogen bonds between the guests and cage (N–H…N$_{py}$ and Cl…H–C$_{py}$) as well as a metal–metal interaction between the platinum atoms of the guests were observed (Fig. 3a).\textsuperscript{11d,16b,16b}

A similar 1H NMR experiment with cisplatin and [Pd$_2$(3A-tripy)$_4$]$^{4+}$ indicated that the 3-amino substituted cage is also able to bind cisplatin in solution, albeit more weakly ($\Delta \delta = 0.07$ ppm for H$_c$, Fig. 4c and d) than the parent [Pd$_2$(tripy)$_4$]$^{4+}$ cage. Conversely, 1H NMR spectra of the [Pd$_2$(2A-tripy)$_4$]$^{4+}$ cage in the presence of cisplatin acquired in either deuterated CH$_3$CN or DMF solvents displayed no shifts relative to the free cage indicating that the 2-amino substituted cage is not able to bind cisplatin. We have previously shown that cisplatin binding is very weak\textsuperscript{12} and that subtle changes to the size, steric profile and electronic properties of the cage cavity\textsuperscript{21} are enough to completely turn off cisplatin binding. Presumably, the lack of cisplatin binding in this system can be ascribed to two factors. Firstly, the presence of the eight amino groups on the exo faces of the cage has caused a twisting of the architecture (as indicated in the crystal structure, Fig. 3, and discussed and listed in Table 1). This twisting subtly alters both the size of the cisplatin binding cavity and the orientations of the hydrogen-bond acceptors and donors groups within the cage cavity, weakening the interaction between the host and the cisplatin guest. Secondly, the electron donating 2-amino units push electron density back onto the terminal pyridyl rings of the tripy ligand. This would reduce the polarisation of the acidic H$_c$ protons of the pyridyl unit, weakening the hydrogen bonding interaction with chloride ligands of the cisplatin guest. These effects, in concert, appear to be enough to fully circumscribe the already weak cisplatin–cage interaction.\textsuperscript{22}

Cytotoxicity

While the improvement of kinetic stability of the [Pd$_2$(2A-tripy)$_4$]$^{4+}$ cage has been achieved at the expense of host–guest capacity, recent work has shown that [Pd$_2$(L)$_4$]$^{4+}$ architectures can act as biological agents in their own right.\textsuperscript{11b,23} Accordingly, a preliminary investigation of the cytotoxic properties of the three cages (and the corresponding ligands) was undertaken against two cell lines: A549 (lung cancer) and cisplatin-resistant MDA-MB-231 (breast cancer) (Table 3). We have previously determined the IC$_{50}$ values for cisplatin against these two cell lines, which were 9.4 ± 0.3 µM (A549)\textsuperscript{22} and 41.2 ± 3.9 µM (MDA-MB-231)\textsuperscript{11b} respectively. None of the ligands exhibited significant cytotoxicity (IC$_{50} > 200$ µM) against these cell-lines. Similarly, neither of the un stabilised cages, [Pd$_2$(tripy)$_4$][BF$_4$]$_4$ or [Pd$_2$(3A-tripy)$_4$][BF$_4$]$_4$ (IC$_{50} > 50$ µM) nor the palladium(u) tetrafluoroborate salt ([Pd$_2$(CH$_3$CN)$_4$][BF$_4$]$_4$) displayed any appreciable cytotoxic effect. The most kinetically stable cage [Pd$_2$(2A-tripy)$_4$][BF$_4$]$_4$ displayed low cytotoxicity against the A549 cell line (IC$_{50} > 50$ µM), and slightly higher cytotoxicity against the

| Table 3 | IC$_{50}$ values for [Pd$_2$(L)$_4$]$^{4+}$ and related literature [Pd$_2$(L)$_4$]$^{4+}$ compounds against selected cell lines |
| --- | --- | --- | --- | --- | --- |
| | A549 (lung) | MDA-MB-231 (breast) | HL-60 (leukemia) | SKW-3 (leukemia) |
| **Compound** | **Ligand** | **Cage** | **Ligand** | **Cage** | **Ligand** | **Cage** | **Ligand** | **Cage** |
| [Pd$_2$(tripy)$_4$][BF$_4$]$_4$ | >200 | >50 | >200 | >50 | — | — | — | — |
| [Pd$_2$(2A-tripy)$_4$][BF$_4$]$_4$ | >200 | >50 | >200 | >50 | — | — | — | — |
| [Pd$_2$(3A-tripy)$_4$][BF$_4$]$_4$ | >200 | >50 | >200 | >50 | — | — | — | — |
| [Pd$_2$(L)$_4$][BF$_4$]$_4$ (L$_4$ = hextrz)$^{11a}$ | 28.5 ± 2.6 | 6.9 ± 0.9 | 89.8 ± 10.7 | 7.0 ± 0.6 | — | >100 | 1.9 ± 0.2 | >80 | 1.8 ± 0.2 |
| [Pd$_2$(L)$_4$][BF$_4$]$_4$ (L$_4$ = dipy-dianthracenyl)$^{23}$ | — | 9.4 ± 0.3 | 41.2 ± 3.9 | 8.1 ± 1.4 | — | 9.3 ± 2.1 | — | — | — |
| Cisplatin | — | — | — | — | 9.4 ± 0.3 | 41.2 ± 3.9 | 8.1 ± 1.4 | 9.3 ± 2.1 | — | — | — |

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MDA-MB-231 cell line (IC$_{50} = 36.4 \pm 1.9 \mu M$). We have previously shown that a more kinetically robust and hydrophobic [Pd$_2$(L)$_4$]$_{2+}$ helicate (L$_4$ = 1,3-bis(1-hexyl-1H,1,2,3-triazol-4-yl)benzene) was considerably more cytotoxic (IC$_{50} = 6.9 \pm 0.9$ and $6.0 \pm 0.6 \mu M$) against both the A549 and MDA-MB-231 cell lines. Additionally, Yoshizawa and co-workers have recently reported that a hydrophobic [Pd$_2$(L)$_4$]$_{4+}$ cage (L$_5$ = 3,3’-[4-(5,6-tris(2-methoxyethoxy)-1,3-phenylene)bis(anthracene-10,9-diyl)]-dipyridine) is highly cytotoxic (IC$_{50} = 1.8$ and $1.9 \mu M$) against HL-60 and SKW-3 cancer cell lines. Thus it is presumed that low cytotoxicity of the systems described here is connected to the combination of both high kinetic lability and the more hydrophilic nature of the [Pd$_2$(L$_{tripy}$)$_4$]$_{4+}$architectures and ligands.

Conclusions

Two amino substituted tripyridyl 2,6-bis(pyridin-3-yethyl) pyridine (tripy) ligands (with amino groups either in the 2-(2A-tripy) or 3-(3A-tripy) positions of the terminal pyridines) and their respective [Pd$_2$(L$_{tripy}$)$_4$]$_{4+}$ cages were synthesised. These systems have been characterised by $^1$H, $^{13}$C and DOSY NMR spectroscopies, high resolution electrospray mass spectrometry, elemental analysis and, in one case, by X-ray crystallography. It was established, using palladium(n) NHC carbene probe model complexes, that the amino substituted compounds were moderately stronger donor ligands than the parent pyridyl system [2A-tripy > 3A-tripy > tripy]. Competition experiments with common biological nucleophiles (Cl$^-$, $^3$H and cyclopentadienyl) showed that the [Pd$_2$(2A-tripy)$_4$]$_{4+}$ cage proved the most kinetically stable, presumably due to a favourable combination of enhanced ligand donor strength, and probably more importantly, intramolecular hydrogen bonding and steric shielding. Preliminary biological investigations against two cell lines (A549 and MDA-MB-231) found that all ligands and cages had IC$_{50}$ values >50 $\mu M$, with the exception of [Pd$_2$(2A-tripy)$_4$]$_{4+}$ which against MDA-MB-231 has an IC$_{50}$ of 36.4 $\pm 1.9 \mu M$.

However, while the ligand tuning resulted in more robust [Pd$_2$(L$_{tripy}$)$_4$]$_{4+}$ architectures the half-lives of the systems against the strongest nucleophiles were still modest ($t_{1/2} = 2-3$ h). Furthermore, the subtle structural changes in the most stabilised cage, [Pd$_2$(2A-tripy)$_4$]$_{4+}$ were found to completely destroy the ability of the system to bind cisplatin. Thus, it appears that in order to exploit these types of metallosupramolecular cage architectures as drug delivery vectors, systems assembled from more kinetically inert metals ions such as Pt(n)$_{16}$ and Ru(n)$_{16}$-Z and Co(m)$_{25}$ will be required. Efforts to generate more robust systems, composed of kinetically inert metal ions, capable of binding drug molecules are underway.

Experimental

General

Unless otherwise stated, all reagents were purchased from commercial sources and used without further purification. 2,5-Dibromo-4-(hydroxymethyl)pyridine, 5-ido-2-aminopyridine, the dimeric dibromobis(benzimidazol-2-ylidene)dipalladium(n) complex, and trans-dibromobis(1,3-diisopropylbenzimidazol-2-ylidene)pyridinepalladium(n) were synthesised according to literature procedures. Solvents were laboratory reagent grade. Petroleum ether refers to the fraction of petrol boiling in the range 40–60 °C, isopropyl alcohol (IPA), methanol (CH$_3$OH), dichloromethane (CH$_2$Cl$_2$), ethylenediaminetetraacetate (EDTA), ethynyltrimethylsilane (TMS-acetylene), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), dimethyl-formamide (DMF). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz Varian 400-MR or Varian 500 MHz AR spectrometer. Chemical shifts are reported in parts per million and referenced to residual solvent peaks (CDCl$_3$: $^1$H δ 7.26 ppm, $^{13}$C δ 77.16 ppm; CD$_3$CN: $^1$H δ 9.4, $^{13}$C δ 132, 1.1 ppm, 118.26 ppm, $d_{4}$-DMSO: $^1$H δ 2.5 ppm, $^{13}$C δ 39.52 ppm). Coupling constants (J) are reported in Hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: m = multiplet, q = quartet, t = triplet, dt = double triplet, d = doublet, dd = double doublet, s = singlet, br = broad. Full $^1$H and $^{13}$C NMR spectra, together with structural labelling are included in the ESI.$^\dagger$ IR spectra were recorded on a Bruker ALPHA FT-IR spectrometer with an attached ALPHA-P measurement module. Microanalyses were performed at the Campbell Microanalytical Laboratory at the University of Otago. Electrospray mass spectra (ESMS) were collected on a Bruker microTOF-Q spectrometer.

Synthesis of 5-iodopyridin-2-2-acetamide (1). A solution of 5-iodopyridin-2-2-amino (2.5 g, 11.4 mmol) in triethylamine (10 mL) and CH$_2$Cl$_2$ (50 mL) was degassed with N$_2$ for 15 minutes. Acetic anhydride (10.7 mL, 110 mmol) was added and the mixture was stirred at room temperature for 18 hours. After removal of solvents under vacuum, the crude mixture was dissolved in 3 : 1 CHCl$_3$/IPA (150 mL), and washed with saturated aqueous NaHCO$_3$ solution (2 × 75 mL), and brine (75 mL). The organic layer was dried over MgSO$_4$, filtered and the solvent removed under vacuum. Column chromatography on silica (1 : 19 acetone/CH$_2$Cl$_2$) gave the product as a brown solid (2.3 g, 8.8 mmol, 77%). $^1$H NMR (400 MHz, $d_6$ -DMSO, 298 K) δ: 10.58 (1H, s, H$_{N=}$), 8.50 (1H, d, $J$ = 2.3 Hz, H$_a$), 8.07 (1H, d, $J$ = 8.8 Hz, H$_b$), 7.94 (1H, d, $J$ = 8.8 Hz, H$_g$), 7.08 (3H, s, H$_d$), 1.32, 151.2, 145.8 (C$_b$), 115.3 (C$_e$), 85.7, 23.9 (C$_g$). HR ESI-MS (CHCl$_3$)/m/z = 284.5 [M + Na]$^+$ (calc. For C$_7$H$_7$I$_2$N$_2$O, 284.95). IR: ν (cm$^{-1}$) 3215, 3139, 3079, 3018, 1767, 1660, 1520, 1362, 1298, 829, 799. Calc. for: C$_7$H$_7$I$_2$N$_2$O: C, 32.08; H, 2.69; N, 10.69%. Found: C, 32.30; H, 2.55; N, 10.71%.
The solution was stirred with aqueous 0.1 M EDTA/NH₂OH solution (40 mL) for 1.5 hours. After washing with aqueous 0.1 M EDTA/NH₂OH (50 mL) and brine (100 mL), the organic layer was dried with Na₂SO₄, filtered, and the solvent removed under vacuum. The solid was purified through column chromatography on silica (1:19 acetone/CH₂Cl₂), giving the product as a brown solid (0.86 g, 3.72 mmol, 97%). ¹H NMR (500 MHz, CDCl₃, 298 K) δ: 8.34 (1H, d, J = 1.6 Hz, Hd), 8.17 (1H, d, J = 8.7 Hz, Hg), 8.07 (1H, br, HNH), 7.77 (1H, dd, J = 8.7 Hz, 2.1 Hz, Hb), 7.41 (2H, ddd, J = 8.6 Hz, 0.8 Hz, 2.3 Hz, Hb), 6.46 (1H, dd, J = 8.6 Hz, 0.8 Hz, Hg), 4.69 (2H, br, HNH), 3.06 (s, Hb).

¹³C NMR (125 MHz, CDCl₃, 298 K) δ: 157.6, 151.6 (C_c), 141.0 (C_b), 130.7, 126.0 (C_b), 124.1 (C_b), 120.3, 101.9, 97.6, 0.0 (C_d). HR ESI-MS (acetone) m/z = 255.09 [M + Na]⁺ (calc. for C₇H₆N₂·0.1 acetone: C, 70.74; H, 5.37; N, 22.60%). Found: C, 70.74; H, 5.17; N, 22.60%.

Synthesis of 5-ethynylpyridin-3-amine (5). A solution of 2 (0.63 g, 2.70 mmol) and NaOH (1.08 g, 27.0 mmol) in methanol (30 mL) was heated at reflux for 1.5 hours before removal of solvent under vacuum, with the resultant residue taken up in 3:1 CHCl₃/IPA (100 mL) and washed with water (100 mL) and brine (100 mL). After drying with Na₂SO₄ and filtration, the solvent was removed under vacuum. Purification of the resultant solid was done by column chromatography on silica (1:8 acetone/CH₂Cl₂) to give the product as a brown solid (0.86 g, 3.72 mmol, 97%). ¹H NMR (500 MHz, CDCl₃, 298 K) δ: 8.13 (1H, d, J = 2.7 Hz, Hg), 8.05 (1H, d, J = 2.7 Hz, Hg), 7.06 (1H, dd, J = 2.7 Hz, 2.7 Hz, Hg), 3.71 (2H, br, HNH), 3.14 (1H, s, Hb).

¹³C NMR (125 MHz, CDCl₃, 298 K) δ: 143.0 (C_c), 141.8, 137.1 (C_b), 124.0 (C_b), 119.1, 80.7, 79.8 (C_d). IR: ν (cm⁻¹) 3328, 3295, 3163, 3020, 2148, 1644, 1585, 1424, 1329, 1246. Anal. calc for C₇H₆N₂·0.1 acetone: C, 70.74; H, 5.37; N, 22.60%. Found: C, 70.96; H, 5.14; N, 22.80%. Mass spectral analysis was not successful for this compound.

**Synthesis of tripy.** A round bottom flask containing (2,6-dibromopyridin-4-yl)ethanol (336 mg, 1.26 mmol), 3-ethylpyridine (325 mg, 3.15 mmol), Cu (24 mg, 0.12 mmol) and Pd[PPh₃]₂Cl₂ (35 mg, 0.05 mmol) was purged with N₂ before addition of triethylamine (25 mL) and dry THF (25 mL). The reaction was stirred in the absence of light for 24 hours. CH₂Cl₂ (50 mL) and aqueous 0.1 M EDTA/NH₂OH solution (100 mL) were added and the mixture stirred at room temperature for 2 hours. After extraction into CH₂Cl₂ (2 × 100 mL), the combined organic layers were washed with brine (100 mL), dried over MgSO₄, and then filtered. The solvent was removed under vacuum. Column chromatography (2:3 CH₂Cl₂/acetonitrile) gave the product as a colourless solid (280 mg, 0.90 mmol, 71%). ¹H NMR (500 MHz, CD₂CN, 298 K) δ: 8.81 (2H, dd, J = 2.1 Hz, 0.8 Hz, Hg), 8.61 (2H, dd, J = 4.9 Hz, 1.7 Hz, Hb), 7.98 (2H, ddd, J = 7.9 Hz, 2.2 Hz, 1.7 Hz, Hb), 7.58 (2H, t, J = 0.9 Hz, Hb), 7.41 (2H, ddd, J = 7.9 Hz, 4.9 Hz, 0.9 Hz, Hb), 4.67 (2H, dt, J = 5.9 Hz, 0.8 Hz, Hb), 3.55 (1H, t, J = 5.9 Hz, HbOH).

¹³C NMR (500 MHz, CD₂CN, 298 K) δ: 154.1, 153.3 (C_c), 150.6 (C_c), 143.8, 139.9 (C_b), 125.3 (C_b), 124.4 (C_b), 119.9, 91.8, 86.2, 62.4 (C_d). HR ESI-MS (acetone) m/z = 645.21 [2M + Na]⁺ (calc. for C₂₀H₁₄N₃NaO₂, 645.20). Found: C, 76.48; H, 4.49; N, 12.90%. Calc. for C₂₀H₁₄N₃NaO₂: C, 76.70; H, 4.49; N, 13.06%.

**Synthesis of 2A-tripy.** In a round bottom flask, diisopropylamine (20 mL) and THF (20 mL) were degassed with N₂ before addition of 3 (400 mg, 3.39 mmol), (2,6-dibromopyridin-4-yl)ethanol (362 mg, 1.35 mmol), Cu (25 mg, 0.14 mmol), and Pd[PPh₃]₂Cl₂ (38 mg, 0.050 mmol) against a positive N₂ flow. The reaction was stirred for 1 h. After filtration, the solvent was removed under vacuum, and the resultant solid was purified by column chromatography on silica (1:8 acetone/CH₂Cl₂) to give the product as a brown solid (0.25 g, 2.1 mmol, 96%). ¹H NMR (500 MHz, CDCl₃, 298 K) δ: 8.27 (2H, d, J = 5.9 Hz, Hg), 8.11 (2H, d, J = 4.9 Hz, Hb), 7.98 (2H, ddd, J = 7.9 Hz, 4.9 Hz, 0.9 Hz, Hb), 4.67 (2H, dt, J = 5.9 Hz, 0.8 Hz, Hb), 3.55 (1H, t, J = 5.9 Hz, HbOH).

¹³C NMR (500 MHz, CD₂CN, 298 K) δ: 154.1, 153.3 (C_c), 150.6 (C_c), 143.8, 139.9 (C_b), 125.3 (C_b), 124.4 (C_b), 119.9, 91.8, 86.2, 62.4 (C_d). HR ESI-MS (acetone) m/z = 645.21 [2M + Na]⁺ (calc. for C₂₀H₁₄N₃NaO₂, 645.20). Found: C, 76.48; H, 4.49; N, 12.90%. Calc. for C₂₀H₁₄N₃NaO₂: C, 76.70; H, 4.49; N, 13.06%.
Synthesis of 3A-tripy. In a round bottom flask, diisopropylamine (20 mL) and THF (20 mL) were degassed with N2 before being added to a solution of diisopropylamine (20 mL) and THF (20 mL). The solution was heated at 40 °C for 48 hours. After removal of the solvent under vacuum, the resultant solid was taken in 3:1 CHCl3/IPA (150 mL) and THF (20 mL) were degassed with N2, before removal of the solvent under vacuum. The resultant solid was suspended in CH2Cl2 (150 mL) and stirred for 40 minutes. Vapor diffusion with diethyl ether in a solution of MeCN. X-ray data were collected at 100(1) K on an Agilent Technologies Supernova system using Cu Kα radiation with 0.03 mmol, 75%).

Synthesis of [Pd2(tripy)4][BF4]4. A solution of 3A-tripy (60 mg, 0.18 mmol) and [Pd(CH3CN)4][BF4]4 (39 mg, 0.090 mmol) in d6-DMDSO (0.75 mL) was sonicated for five minutes. Additon of ethyl acetate (20 mL) resulted in precipitation of the product. The precipitate was collected by filtration, and washed with ethyl acetate (15 mL) and diethyl ether (15 mL) to give a brown solid (64 mg, 0.03 mmol, 75%). OR A solution of 3A-tripy (60 mg, 0.18 mmol) and [Pd(CH3CN)4][BF4]4 (39 mg, 0.090 mmol) in DMF (2 mL) was sonicated for five minutes. Additon of ethyl acetate (20 mL) resulted in precipitation of the product. The precipitate was collected by filtration, and washed with ethyl acetate (15 mL) and diethyl ether (15 mL) to give a brown solid (64 mg, 0.03 mmol, 75%). OR A solution of 3A-tripy (60 mg, 0.18 mmol) and [Pd(CH3CN)4][BF4]4 (39 mg, 0.090 mmol) in DMF (2 mL) was sonicated for five minutes. Additon of ethyl acetate (20 mL) resulted in precipitation of the product. The precipitate was collected by filtration, and washed with ethyl acetate (15 mL) and diethyl ether (15 mL) to give a brown solid (64 mg, 0.03 mmol, 75%).
solvent lattice were severely disordered and could not be appropriately modelled. The SQUEEZE routine within PLATON was employed to resolve this problem, resulting in ten void spaces (total of 460 electrons), variously assigned to tetrafluoroborate anions (8 in total), H2O (5 in total) and MeCN (3 in total) solvent molecules (total of 444 electrons), as described below. Despite repeated efforts (>50 crystallisations and data collections over a two year period) to crystallise the compound, the most suitable candidate was small and a poor diffractor. The data quality is poor, with two A alerts (a large Hirshfield difference and high MainVol $U_{eq}$, compared to neighbours) and many B alerts, and we emphasise that metric data cannot be reliably extracted from the structure and should be treated with caution. However, the connectivity of the cationic framework is readily apparent. The methylene alcohol substituents from the four neighbouring cages in the lattice interpenetrate the cavity of each cage [Fig. 3 and ES1†], interpenetrating groups shown in spacefilling mode (yellow, green, blue and pink) and preclude cisplatin encapsulation in the solid state. Around the coordinating pyridine rings, the amino groups form a hydrogen bonding network. SQUEEZE details and crystallographic parameters can be found in the ES1†.

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