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

Dan Preston, a Samantha M. McNeill, b James E. M. Lewis, a Gregory I. Giles b and James D. Crowley a

There is considerable interest in exploiting metallosupramolecular cages as drug delivery vectors. Recently, we developed a \([\text{Pd}_2\text{L}_4]^{4+}\) 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 \([\text{Pd}_2\text{L}_{\text{tripy}}]^{4+}\) cages. These systems have been characterised by 1H, 13C 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 \([\text{Pd}_2(2\text{A-tripy})]^{4+}\) proving the most stable. Biological testing on the three ligands and cages against A549 and MDA-MB-231 cell lines showed that only \([\text{Pd}_2(2\text{A-tripy})]^{4+}\) exhibited any appreciable cytotoxicity, with a modest IC₅₀ of 36.4 ± 1.9 µM against the MDA-MB-231 cell line. Unfortunately, the increase in kinetic stability of the \([\text{Pd}_2(\text{L}_{\text{tripy}})]^{4+}\) 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) \([\text{Pd}_2\text{L}_3]^{4+}\) cage capable of binding cisplatin (cis-[Pt(NH₃)₂Cl₂]). We hoped to exploit these \([\text{Pd}_2\text{L}_4]^{4+}\) cages as metallosupramolecular drug delivery vectors, unfortunately the cage architecture was rapidly decomposed when exposed to chloride (Cl⁻), histidine (his) and cysteine (cys) (common nucleophiles in biological systems). In order to use these cisplatin binding \([\text{Pd}_2\text{L}_4]^{4+}\) 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 tripyrdyl ligands in an effort to obtain more kinetically robust \([\text{Pd}_2\text{L}_4]^{4+}\) 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 \(\text{tripy}, \ 2\text{A-tripy}, \text{and} \ 3\text{A-tripy} \) (Scheme 1) were synthesised using standard methods\(^{11a,d,12,14}\) (ESI†) and characterised using NMR spectroscopy, high resolution electrospray mass spectrometry (HR-ESMS), and elemental analysis (Experimental section and ESI†).

Addition of one of the ligands \(\text{tripy}, \ 2\text{A-tripy}, \text{and} \ 3\text{A-tripy}\) to a solution of \([\text{Pd}(\text{CH}_3\text{CN})_4]_2(\text{BF}_4)_2\) in a 2 : 1 ratio resulted in the formation of the desired cages (Scheme 1). While the \text{tripy}\) and \(3\text{A-tripy}\) containing cages formed instantaneously at room temperature, the formation of \([\text{Pd}_2(2\text{A-tripy})_4]_4(\text{BF}_4)_4\) required heating in DMSO solution at 50 °C for six hours to complete the assembly. The cages were characterised using \(^1\text{H}\) and DOSY NMR spectroscopy, HR-ESMS, and elemental analysis, (Experimental and ESI†). The \(^1\text{H}\) NMR spectra (Fig. 1 and ESI†) of the \([\text{Pd}_2(L_{\text{tripy}})_4]_4^{4+}\) cages show a single set of peaks. The proton resonances due to the terminal pyridyl units of the cages (Hc) are shifted downfield (\(\Delta\delta = 0.19-0.59 \text{ ppm}\)) relative to the free \text{tripy}\) ligands, consistent with complexation to palladium(II) ions. Interestingly, the proton resonance of the 2-amino group in the \([\text{Pd}_2(2\text{A-tripy})_4](\text{BF}_4)_4\) cage displays a very large \(\Delta\delta = 1.56 \text{ ppm}\) 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 \([\text{Pd}_2(3\text{A-tripy})_4](\text{BF}_4)_4\) cage is much smaller \(\Delta\delta = 0.81 \text{ ppm}\) (ESI†). The sharp, uncomplicated signals observed in the \(^1\text{H}\) NMR spectra of the \([\text{Pd}_2(L_{\text{tripy}})_4]_4^{4+}\) systems are similar to what was previously observed for the formation of other \([\text{Pd}_2(L)_4]_4^{4+}\) cages and are consistent with the formation of complexes of high symmetry in solution.\(^{11a,d,12}\)

Diffusion-ordered \(^1\text{H}\) NMR spectroscopy (DOSY) provided additional strong support for the selective formation of the cages in solution. \(^1\text{H}\) DOSY spectra (d6-DMSO, 298 K) were obtained for ligands \(\text{tripy}, \ 2\text{A-tripy}, \text{and} \ 3\text{A-tripy}\) and cages \([\text{Pd}_2(\text{tripy})_4]_4^{4+}\), \([\text{Pd}_2(2\text{A-tripy})_4]_4^{4+}\) and \([\text{Pd}_2(3\text{A-tripy})_4]_4^{4+}\) (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 \(D_{\text{complex}}/D_{\text{ligand}}\) ratios of \(\sim0.50:1\) 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†).

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 [PdL\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†). 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+} cation confirms that the coordination of the 2-amino pyridyl units to the palladium(II) ions was monodentate through the pyridyl nitro-
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 2  pK_a values, ¹³C NMR chemical shifts of NHC carbene of the model probe complexes, and half-lives (t_1/2) for the decomposition of the [Pd₂(tripy)₄]⁺⁺⁺ architectures against selected biologically relevant nucleophiles (3:2 d₆-DMSO/D₂O, 298 K, 500 MHz) as measured through time-course ¹H NMR spectroscopy

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK_a (4 eq.)</th>
<th>¹³C δ (8 eq.)</th>
<th>Cl⁻ (4 eq.)</th>
<th>his (4 eq.)</th>
<th>cys (4 eq.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Pd₂(tripy)₄][BF₄]₄</td>
<td>5.23⁺⁺⁺</td>
<td>159.3⁺⁺⁺⁺</td>
<td>&gt;1 min</td>
<td>18 min</td>
<td>6 min</td>
</tr>
<tr>
<td>[Pd₂(2A-tripy)₄][BF₄]₄</td>
<td>6.82⁺⁺⁺</td>
<td>161.2⁺⁺⁺⁺</td>
<td>2 h</td>
<td>46 h</td>
<td>3 h</td>
</tr>
<tr>
<td>[Pd₂(3A-tripy)₄][BF₄]₄</td>
<td>6.04⁺⁺⁺⁺</td>
<td>159.8⁺⁺⁺⁺</td>
<td>10 min</td>
<td>25 min</td>
<td>10 min</td>
</tr>
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</table>

¹ Chemical shift (ppm) of the NHC carbene carbon in the model Pd(II) probe complexes (ESI).

The kinetic stability of the [Pd₂(Ltripy)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-DMSO/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]⁺⁺⁺ cage was rapidly decomposed by all the nucleophiles. The half-life for the decomposition of the [Pd₂(tripy)₄]⁺⁺⁺ complex with his was 18 minutes. Despite the [Pd₂(3A-tripy)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]⁺⁺⁺ 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]⁺⁺⁺ 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]⁺⁺⁺ 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]⁺⁺⁺ are less than 3 h suggesting that these systems would need further tuning in order to be useful in a biological setting.

Cisplatin binding

We¹⁴,¹²,¹⁹ and others¹⁶ have previously shown that other similar [Pd₂(tripy)₄]⁺⁺⁺ cages can encapsulate cisplatin through hydrogen bonding interactions in CH₃CN and DMF solvents.²⁰
1H NMR spectroscopy and recently reported crystallographic evidence confirms that despite the presence of the potential hydrogen bond donor CH$_2$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 of the [(cisplatin)$_3$C(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. Consistent with the 1H NMR evidence, the [Pd$_2$(tripy)$_4$]$^{4+}$ cation binds two molecules of cisplatin within the cavity of the cage. 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). 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 and that subtle changes to the size, steric profile and electronic properties of the cage cavity 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.

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. 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 $\pm$ 0.3 $\mu$M (A549) and 41.2 $\pm$ 3.9 $\mu$M (MDA-MB-231), respectively. None of the ligands exhibited significant cytotoxicity (IC$_{50}$ > 200 $\mu$M) against these cell lines. Similarly, neither of the unstabilised cages, [Pd$_2$(tripy)$_4$][BF$_4$]$_4$ or [Pd$_2$(3A-tripy)$_4$][BF$_4$]$_4$ (IC$_{50}$ > 50 $\mu$M) nor the palladium(tetrafluoroborate salt (IC$_{50}$ > 100 $\mu$M))]$_{11d}$ 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 $\mu$M), and slightly higher cytotoxicity against the

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<tr>
<th>Table 3</th>
<th>IC$_{50}$ values for [Pd$_2$(L)$_4$]$^{4+}$ and related literature [Pd$_2$(L)$_4$]$^{4+}$ compounds against selected cell lines</th>
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</thead>
<tbody>
<tr>
<td>Compound</td>
<td>IC$_{50}$ (µM)</td>
</tr>
<tr>
<td></td>
<td>A549 (lung)</td>
</tr>
<tr>
<td>[Pd$_2$(tripy)$_4$][BF$_4$]$_4$</td>
<td>&gt;200</td>
</tr>
<tr>
<td>[Pd$_2$(2A-tripy)$_4$][BF$_4$]$_4$</td>
<td>&gt;200</td>
</tr>
<tr>
<td>[Pd$_2$(3A-tripy)$_4$][BF$_4$]$_4$</td>
<td>&gt;200</td>
</tr>
<tr>
<td>[Pd$_2$(L)$_4$][BF$_4$]$_4$ (L$<em>a$ = hextrz)$</em>{11d}$</td>
<td>28.5 $\pm$ 2.6</td>
</tr>
<tr>
<td>[Pd$_2$(L)$_4$][BF$_4$]$_4$ (L$<em>a$ = dipy-dianthracenyl)$</em>{23}$</td>
<td>—</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>—</td>
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MDA-MB-231 cell line (IC\textsubscript{50} = 36.4 ± 1.9 µM). We have previously shown that a more kinetically robust and hydrophobic [Pd\textsubscript{2}(\textit{L\textsubscript{a}})]\textsuperscript{4+} helicate (IC\textsubscript{50} = 1.3±1-3-hexyl-1H-1,3-triazol-4-yl) benzene was considerably more cytotoxic (IC\textsubscript{50} = 6.9 ± 0.9 and 6.0 ± 0.6 µM) against both the A549 and MDA-MB-231 cell lines.\textsuperscript{11a} Additionally, Yoshizawa and co-workers have recently reported that a hydrophobic [Pd\textsubscript{2}(\textit{L\textsubscript{b}})]\textsuperscript{4+} cage (IC\textsubscript{50} = 3.3±((4,5,6-tris(2-methoxyethoxy)-1,3-phenylene)bisisanthracene-10,9-diyl) dipyrindine) is highly cytotoxic (IC\textsubscript{50} = 1.8 and 1.9 µM) against HL-60 and SKW-3 cancer cell lines.\textsuperscript{23} 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 hydrophobic nature of the [Pd\textsubscript{2}(\textit{L\textsubscript{cyp}})]\textsuperscript{4+} architectures and ligands.

### Conclusions

Two amino substituted tripyridyl 2,6-bis(pyridin-3-ylethynyl) pyridine (\textit{tripy}) ligands (with amino groups either in the 2-(\textit{2A-tripy}) or 3-(\textit{3A-tripy}) positions of the terminal pyridines) and their respective [Pd\textsubscript{2}(\textit{L\textsubscript{tripy}})]\textsuperscript{4+} cages were synthesised. These systems have been characterised by \textit{H}, \textit{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 (2\textit{A-tripy} > 3\textit{A-tripy} > \textit{tripy}). Competition experiments with common biological nucleophiles (\textit{Cl}\textgreek{g}, \textit{his} and cys) showed that the [Pd\textsubscript{2}(\textit{2A-tripy})]\textsuperscript{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\textsubscript{50} values >50 µM, with the exception of [Pd\textsubscript{2}(\textit{2A-tripy})]\textsuperscript{4+} which against MDA-MB-231 has an IC\textsubscript{50} of 36.4 ± 1.9 µM.

However, while the ligand tuning resulted in more robust [Pd\textsubscript{2}(\textit{Ltripy})]\textsuperscript{4+} architectures, the half-lives of the systems against the stronger nucleophiles were still modest (\textit{t}_{1/2} = 2–3 h). Furthermore, the subtle structural changes in the most stabilised cage, [Pd\textsubscript{2}(\textit{2A-tripy})]\textsuperscript{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)\textsuperscript{26} and Ru(n)\textsuperscript{27,28} and Co(n)\textsuperscript{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,\textsuperscript{26} 5-ido-2-aminopyridine,\textsuperscript{6a} the dimeric dibromobis(benzimidazolin-2-ylidene) palladium(n) complex,\textsuperscript{27} and \textit{trans}-dibromo(1,3-diisopropylbenzimidazolin-2-ylidene)(pyridine)palladium(n)\textsuperscript{16b} 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\textsubscript{3}OH), dichloromethane (CH\textsubscript{2}Cl\textsubscript{2}), ethylenediaminetetraacetate (EDTA), ethynyltrimethylsilane (TMS-acetylene), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), dimethylformamide (DMF). \textit{H} and \textit{C} NMR spectra were recorded on either a 400 MHz Varian 400-kr or Varian 500 MHz AR spectrometer. Chemical shifts are reported in parts per million and referenced to residual solvent peaks (CDCl\textsubscript{3}: \textit{1H} δ 7.26 ppm, \textit{13C} δ 77.16 ppm; CD\textsubscript{3}CN: \textit{1H} δ 1.94, \textit{13C} δ 132.1, 118.26 ppm, \textit{d}_{6}-DMSO: \textit{1H} δ 2.50 ppm, \textit{13C} δ 39.52 ppm). Coupling constants (\textit{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 \textit{H} and \textit{13C} NMR spectra, together with structural labelling are included in the ESI.† 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-acetamide (1).** A solution of 5-iodopyridin-2-amine (2.5 g, 11.4 mmol) in triethylamine (10 mL) was degassed with N\textsubscript{2} for 48 hours. The solvent was removed under vacuum, and the resulting solid was taken up in 3 : 1 CHCl\textsubscript{3}/IPA (40 mL). The organic layer was dried over MgSO\textsubscript{4}, filtered and the solvent removed under vacuum. Column chromatography on silica (1:1 acetone/CH\textsubscript{2}Cl\textsubscript{2}) gave the product as a brown solid (2.3 g, 8.8 mmol, 77%). \textit{H} NMR (400 MHz, \textit{d}_{6}-DMSO, 298 K) δ: 10.58 (1H, s, N\textsubscript{H}, 8.50 (1H, d, \textit{J} = 2.3 Hz, \textit{H}b), 8.07 (1H, d, \textit{J} = 8.8 Hz, \textit{H}b), 7.94 (1H, d, \textit{J} = 8.8 Hz, \textit{H}a), 2.08 (3H, s, \textit{H}a). \textit{13C} NMR (100 MHz, \textit{d}_{6}-DMSO, 298 K) δ: 169.4, 153.3 (\textit{Ca}), 151.2, 145.8 (\textit{Cb}), 115.3 (\textit{Cf}), 85.7, 23.9 (\textit{Cd}). HR ESI-MS (CH\textsubscript{13}Cl\textsubscript{13}O) \textit{m/z} = 284.95 [M + Na]\textsuperscript{+} (calc. For C\textsubscript{7}H\textsubscript{7}I\textsubscript{2}N\textsubscript{2}O, 284.95). IR: ν (cm\textsuperscript{-1}) 3215, 3139, 3079, 3018, 1676, 1660, 1520, 1362, 1182, 1058, 968, 799, 799. Anal. calcd for: C\textsubscript{7}H\textsubscript{7}I\textsubscript{2}N\textsubscript{2}O, C, 32.08; H, 2.69; N, 10.69%. Found: C, 32.30; H, 2.55; N, 10.71%.

**Synthesis of N-(5-((trimethylsilyl)ethyl)pyridin-2-yl)acetamide (2).** A round bottom flask containing 1 (1.00 g, 3.82 mmol), C\textsubscript{u}I (0.07 g, 0.38 mmol) and Pd(PPh\textsubscript{3})\textsubscript{2}Cl\textsubscript{2} (0.19 g, 0.27 mmol) was purged with N\textsubscript{2} for 15 minutes. After adding TMS-acetylene (0.68 g, 9.30 mL, 6.87 mmol) \textit{via} syringe, the reaction was heated at reflux under N\textsubscript{2} for 48 hours. The solvent was removed under vacuum, and the resulting solid was taken up in 3 : 1 CHCl\textsubscript{3}/IPA (40 mL).
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%).

\[ \text{H NMR (500 MHz, CDCl}_3, 298 K) \delta: 8.34 (1H, d, J = 1.6 Hz, H_a), 8.17 (1H, d, J = 8.7 Hz, H_b), 8.07 (1H, br, H_s, H_b), 7.77 (1H, dd, J = 8.7 Hz, 2.1 Hz, H_c), 2.11 (3H, s, H_s). } \]

\[ \text{13C NMR (125 MHz, CDCl}_3, 298 K) \delta: 168.5, 150.8 (C_b), 150.3, 141.4 (C_d), 116.0, 112.9 (C_b), 101.4, 97.1, 24.8 (C_d) -0.1 (C_d). } \]

HR ESI-MS (CHCl₃) m/z = 255.09 [M + Na]⁺ (calc. for C₁₂H₁₆N₂Si·0.5 acetone, 255.09).

IR: ν (cm⁻¹) 3282, 3295, 3020, 2148, 1644, 1585, 1424, 1239, 1246. Anal. calc. for C₁₂H₁₆N₂O·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 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, and purification through column chromatography on silica (1:8 acetone/CH₂Cl₂) gave the product as a brown solid (0.22 g, 1.90 mmol, 70%).

\[ \text{1H NMR (500 MHz, CDCl}_3, 298 K) \delta: 8.13 (1H, dd, J = 2.7 Hz, H_a), 8.05 (1H, d, J = 2.7 Hz, H_b), 7.06 (1H, dd, J = 2.7 Hz, 2.7 Hz, H_c), 3.71 (2H, br, H_s, H_b), 3.14 (1H, s, H_d). } \]

\[ \text{13C NMR (125 MHz, CDCl}_3, 298 K) \delta: 143.0 (C_b), 141.8, 137.1 (C_a), 124.0 (C_d), 119.1, 80.7, 79.8 (C_d). } \]

IR: ν (cm⁻¹) 3328, 3295, 3163, 3020, 2148, 1644, 1585, 1424, 1239, 1246. Anal. calc. for C₁₂H₁₆N₂·0.1 (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)methanol (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. 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/acetone) gave the product as a colourless solid (280 mg, 0.90 mmol, 71%).

\[ \text{1} \text{H NMR (500 MHz, CDCl}_3, 298 K) \delta: 8.21 (2H, dd, J = 2.1 Hz, 0.8 Hz, H_a), 8.61 (2H, dd, J = 4.9 Hz, 1.7 Hz, H_b), 7.98 (2H, d, J = 7.9 Hz, 2.2 Hz, 1.7 Hz, H_a), 7.59 (2H, t, J = 0.9 Hz, H_b), 7.41 (2H, dd, J = 7.9 Hz, 4.9 Hz, 0.9 Hz, H_b), 4.67 (2H, dt, J = 5.9 Hz, 0.8 Hz, H_a). } \]

\[ \text{IR: ν (cm⁻¹) 3328, 3327, 3295, 3243, 2955, 2161, 1697, 1597, 1526, 1536, 1303, 1246, 1030. Anal. calc. for C₂₀H₁₃N₃NaO, 334.10). IR: ν (cm⁻¹) 3282, 3295, 3163, 3142, 1654, 1585, 1424, 1329, 1165. Anal. calc. for C₁₂H₁₆N₂Si·0.5 acetone: C, 70.74; H, 5.37; N, 22.60%. Found: C, 70.44; H, 5.17; N, 22.56%. } \]

**Synthesis of N-(5-((trimethylsilyl)ethenyl)pyridin-3-yl)acetamide (4).** A glass tube containing diisopropylpyridine (7 mL) degassed with N₂ was charged with 5-bromopyridin-3-amine (500 mg, 2.89 mmol), CuI (55 mg, 0.29 mmol), Pd(dppf)₂Cl₂ (85 mg, 0.17 mmol) and TMS-acetylene (1.173 mL, 852 mg, 8.67 mmol) against a positive N₂ flow. The tube was sealed and stirred at 60 °C for 72 hours. 3:1 CHCl₃/IPA (100 mL) and aqueous 0.1 M EDTA/NH₂OH solution (100 mL) were added and the mixture stirred for one hour. The organic layer was washed with water (50 mL) and brine (50 mL), dried over Na₂SO₄, filtered, and the solvent was removed under vacuum. The resultant solid was purified by column chromatography (1:8 acetone/CH₂Cl₂) to give the product as a brown solid (512 mg, 2.71 mmol, 94%).

\[ \text{1H NMR (500 MHz, CDCl}_3, 298 K) \delta: 8.10 (1H, d, J = 1.5 Hz, H_a), 8.01 (1H, d, J = 2.7 Hz, H_b), 7.04 (1H, d, J = 2.7 Hz, 1.7 Hz, H_c), 0.25 (9H, s, H_s). } \]

\[ \text{13C NMR (125 MHz, CDCl}_3, 298 K) \delta: 142.9 (C_b), 141.9, 136.8 (C_e), 124.1 (C_d), 120.3, 101.9, 97.6, 0.0 (C_d). } \]

HR ESI-MS (MeOH) m/z = 191.10 [M + H⁺] (calc. for C₅H₆N₂Si, 191.10). Anal. calc. for C₅H₆N₂OSi: C, 63.11; H, 7.41; N, 14.62%; found: C, 63.40; H, 7.48; N, 14.62%. IR: ν (cm⁻¹) 3327, 3295, 3162, 2147, 1585, 1424, 1329, 1245.

**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)methanol (362 mg, 1.35 mmol), CuI (25 mg, 0.14 mmol), and Pd(PPh₃)₂Cl₂ (38 mg, 0.050 mmol) against a positive N₂ flow. The solution was heated at 50 °C for 48 hours. After removal of the solvent under vacuum, the resultant solid was taken up in 3:1 CHCl₃/IPA (150 mL) and aqueous 0.1 M EDTA/NH₂OH solution (50 mL) and stirred for 40 minutes. The organic layer was washed with brine, dried with Na₂SO₄, filtered and then the solvent was removed under vacuum. Purification of the resultant solid on a silica column deactivated with 3:97 triethylamine/CH₂Cl₂ (0.5/4.5/95 then 1/90 saturated aqueous NH₂OH solution/MeOH/CH₂Cl₂) gave the product as a brown solid (364 mg, 1.07 mmol, 79%).
298 K) δ: 8.18 (2H, dd, J = 2.3 Hz, 0.5 Hz, H_d), 7.57 (2H, dd, J = 8.6 Hz, 2.3 Hz, H_c), 7.42 (2H, s, H_e), 6.55 (4H, s, H_NH), 6.49 (2H, dd, J = 8.6 Hz, 0.6 Hz, H_b), 5.51 (1H, t, J = 5.8 Hz, H_OH), 4.54 (2H, d, J = 5.7 Hz, H_d), 5^1^3^C NMR (500 MHz, d_6-DMSO, 298 K) δ: 159.7, 153.0, 152.0 (C_c), 142.9, 139.7 (C_d), 122.6 (C_a), 107.7 (C_e), 104.9, 88.8, 88.0, 60.9 (C_d). HR ESI-MS (CH_3CN) m/z = 683.26 [M + H]^+ (calc. for C_8H_13N_11O_3, 683.26), 342.13 [M + H]^+ (calc. for C_6H_9N_2O_2, 342.14). IR: ν (cm^-1) 3307, 3145, 2921, 2852, 2220, 1670, 1534, 1505, 1393, 1171, 1140, 1055, 1016. Anal. calcld for C_80H_52N_12O_4Pd_2·3H_2O: C, 51.67; H, 3.14; N, 12.03%. Found: C, 51.60; H, 3.12; N, 12.02%.

**Synthesis of 3A-tripy.** In a round bottom flask, diisopropylamine (20 mL) and THF (20 mL) were degassed with N_2, before 5 (365 mg, 3.09 mmol), (2,6-dibromopyridin-4-yl)methanol (275 mg, 1.03 mmol), CuI (20 mg, 0.10 mmol), and Pd(PPh_3)_4 (36 mg, 0.05 mmol) were added and heated under vacuum. The solution was heated at 40 °C for 6 hours. 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%).

**Synthesis of [Pd_2(tripy)_4](BF_4)_4.** A solution of 3A-tripy (60 mg, 0.18 mmol) and [Pd(CH_3CN)_4](BF_4)_2 (39 mg, 0.090 mmol) in d_6-DMSO (0.75 mL) was sonicated for five minutes. Addition 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%).

**Synthesis of [Pd_2(3A-tripy)_4](BF_4)_4.** A solution of 3A-tripy (60 mg, 0.18 mmol) and [Pd(CH_3CN)_4](BF_4)_2 (39 mg, 0.090 mmol) in d_6-DMSO (0.75 mL) was sonicated for five minutes. Addition 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%).

**Synthesis of [Pd_2(2A-tripy)_4](BF_4)_4.** A solution of 2A-tripy (62 mg, 0.20 mmol) and [Pd(CH_3CN)_4](BF_4)_2 (44 mg, 0.10 mmol) in acetonitrile (6 mL) was stirred for 1 hour. Vapor diffusion with diethyl ether gave a white solid (76 mg, 0.040 mmol, 85%).

**Synthesis of [Pd_2(2A-tripy)_4](BF_4)_4.** A solution of 2A-tripy (40 mg, 0.12 mmol) and [Pd(CH_3CN)_4](BF_4)_2 (26 mg, 0.060 mmol) in 1.5 mL d_6-DMSO in a tube was purged with N_2 and was heated at 50 °C for 6 hours. Diethyl ether (30 mL) was added and the solution shaken vigorously. After decanting the liquid portion, the precipitate was suspended in CH_2Cl_2 (5 mL) and isolated by filtration. After washing with CH_2Cl_2 (5 mL), the solid was dried under vacuum at 60 °C for 4 days to give the product as a red solid (32 mg, 0.017 mmol, 57%).
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), H$_2$O (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 MainMoU $U_{eq}$ 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 ESIF), 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 ESIF.$^\dagger$

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**Notes and references**


10 (a) V. Croué, S. Goeb and M. Salle, *Chem. Commun.*, 2015, **51**, 7275–7289; (b) M. Frank, J. Hey, I. Balcioglu, Y.-S. Chen,
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16 (a) While our manuscript was being refereed Casini, Kuhn and co-workers reported the X-ray crystal structure of the cisplatin-[Pd2(tripy)4]4+ host guest adduct, see: (b) A. Schmidt, V. Molano, M. Hollering, A. Pöthig, A. Casini and F. E. Kühn, Chem. – Eur. J., 2016, 22, 2253–2256.


18 (a) Huynh and co-workers have shown that benzimidazol-2-yliden-dibromopalladium(u) complexes can be used to probe the σ-donor strength of the ligands trans to the benzimidazol-2-ylidene. They have found that there is a direct relationship between the σ-donor strength of the trans monodentate ligand and the chemical shift of the benzimidazole carbene carbon in the 13C NMR spectra of the dibromopalladium(u) complexes, see: (b) H. V. Huynh, Y. Han, R. Jothisbasu and J. A. Yang, Organometallics, 2009, 28, 5395–5404.


20 Cisplatin binding within the [Pd2(tripy)4]4+ cage cavity is only observed in CD3CN and DMF-d7. We have generated a water soluble [Pd2(tripy)4]4+ cage using nitrate (NO3−) counter anions and examined the binding of cisplatin in D2O (ESI†). As we have seen previously in D2O (ref. 19a) no host–guest interaction between the cage and cisplatin was observed (ESI†). Similarly, we have also examined the host–guest interaction between the[Pd2(tripy)4]4+ cage and cisplatin in DMSO-d6 (ESI†). As with the D2O experiments no interaction between the cage and the cisplatin was observed. Additionally, the DMSO solvent reacts with cisplatin releasing either (Cl− or NH3) ligands which then react with the [Pd2(tripy)4]4+ cation leading to cage decomposition (ESI†).


22 A referee suggested that the hydrogen bonding between the exo-methylene alcohol unit and the central endo-pyridyl group observed in the crystal structure of [Pd2(2a-tripy)4]4+ could be responsible for the lack of cisplatin binding. We rule that postulate out for the following reasons: (1) 1H NMR and DOSY experiments (DMF-d7, ESI†) suggest that the [Pd2(2a-tripy)4]4+ cage is monomeric in solution. The diffusion coefficient for the [Pd2(2a-tripy)4]4+ cage was essentially identical to that of cage that does not feature the exo-methylene alcohol unit suggesting that those two cages are of very similar size in solution. Additionally, the 1H NMR spectra of the cage are not concentration dependent ruling out any aggregation of the [Pd2(2a-tripy)4]4+ cage into a larger oligomeric or polymeric architectures; (2) All the cages studied have the exo-methylene alcohol unit and the central endo-pyridyl group. Thus if hydrogen bonding between those substituents was a factor it would be expected to interfere with cisplatin binding in all the cage systems. It does not, the [Pd2(2a-tripy)4]4+ both interact with cisplatin in DMF solution.


