Exo-Functionalized Metallacages as Host-Guest Systems for the Anticancer Drug Cisplatin

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Within the framework of designing new self-assembled metallosupramolecular architectures for drug delivery, seven \([\text{Pd}_2\text{L}_4]^4+\) metallacages (\(L = 2,6\text{-bis(pyridine-3-yl ethynyl)pyridine}\)) featuring different groups in exo-position, selected to enhance the cage solubility in aqueous environment, were synthesized. Thus, carboxylic acids, sugars, and PEG groups were tethered to the bispyridyl ligands directly or via disulfide bond formation, as well as via click chemistry. The ligands and respective cages were characterized by different methods, including NMR spectroscopy and high-resolution electrospray mass spectrometry (HR-ESI-MS). While the two ligands featuring carboxylic acid-functionalized groups showed improved solubility in water, the other ligands were soluble only in organic solvents. Unfortunately, all the respective self-assembled cages were also insoluble in water. Afterwards, the encapsulation properties of the anticancer drug cisplatin in selected \([\text{Pd}_2\text{L}_4]\text{X}_4\) cages (\(\text{X} = \text{NO}_3^-, \text{BF}_4^-\)) were studied by \(^1\text{H}, \ ^1\text{H DOSY, and } ^{195}\text{Pt NMR spectroscopy. The effect of the counter ions as well as of the polarity of the solvent in the drug encapsulation process were also investigated, and provided useful information on the host-guest properties of these experimental drug delivery systems. Our results provide further experimental support for previous studies that suggest the desolvation of guests from surrounding solvent molecules and the resulting solvent rearrangement may actually be the primary driving force for determining guest binding affinities in metallacages, in the absence of specific functional group interactions.

Keywords: metallacages, supramolecular coordination complexes, water solubility, host-guest chemistry, cisplatin

INTRODUCTION

Supramolecular chemistry has its roots in biology, and in recent years the unique and often advantageous properties of supramolecular materials have led to their extensive exploration in the fields of biomolecular recognition, drug delivery, disease diagnosis, and imaging. In this context, supramolecular coordination complexes (SCCs) hold great promise (Cook et al., 2013; Casini et al., 2017). In fact, the number of reports on the bioactivity of three-dimensional (3D) SCCs with different shapes has substantially increased (Kaner et al., 2016; Preston et al., 2016; Casini et al., 2017) and includes helicates (Schmitt et al., 2012), metallacages (Vajpayee et al., 2011), cubes (Ahmedova et al., 2016), prisms(Ahmad et al., 2015), and capsules (Therrien, 2013). These systems are of general formula \(\text{M}_n\text{L}_m\), where \(\text{M}\) is usually Fe(II), Pd(II), Pt(II), or half-sandwich organometallic clips based on Ru(II), Os(II), or Ir(III) and Rh(III), and \(\text{L}\) is the ligand of the coordination complex.
In 2012, Lewis et al. reported on the encapsulation properties of the anticancer drug cisplatin within cationic \([\text{Pd}_2\text{L}_4]^{4+}\) cages \((L = 2,6\text{-bis}(\text{pyridine}-3\text{-yethyl})\text{pyridine} \text{as the bidentate ligand})\) studied by NMR and X-ray diffraction analysis (Lewis et al., 2012). Following these promising results, as part of our efforts to develop functional metallosupramolecular drug delivery vectors, we have recently expanded the family of \(exo\)-functionalized \([\text{Pd}_2\text{L}_4]^{4+}\) cages coupled to fluorescent groups for imaging by fluorescence microscopy (Kaiser et al., 2016; Schmidt et al., 2016a,b), as well as to peptides for targeted drug delivery of cisplatin to cancer cells (Han et al., 2017, 2018). It is worth mentioning that while cisplatin occupies a crucial role in the treatment of various malignant tumors, its efficacy, and applicability are heavily restricted by severe systemic toxicities and drug resistance. Thus, our recent studies have exploited the host-guest properties of targeted metallacages to enhance the activity of cisplatin in cancer cells, protecting it from metabolism (Han et al., 2017). Of note, the reduced toxicity of cisplatin encapsulated in integrin targeted metallacages has also been demonstrated by us in an ex vivo model (Han et al., 2017).

As the initially developed cage architectures displayed scarce water solubility, we were interested in improving their hydrophilicity via derivatization of the ligand scaffold. Thus, here, a small library of bis(pyridyl) ligands—of general scaffold 3,5-bis(3-ethynylpyridine)phenyl (Scheme 1) - \(exo\)-functionalized with chemical groups envisioned to improve the aqueous solubility, were synthesized and self-assembled into the corresponding \([\text{Pd}_2\text{L}_4]^{4+}\) cages. The products were characterized by NMR, HR-ESI-MS, and IR. The reduced toxicity of cisplatin encapsulated in integrin targeted metallacages has also been demonstrated by us in an ex vivo model (Han et al., 2017).

RESULTS AND DISCUSSION

Adapting previously reported procedures (Schmidt et al., 2016b), the synthesis of the \(exo\)-functionalized ligand scaffold with carboxylic acid groups, \(L_1\), and \(L_2\) was first achieved by Sonogashira cross-coupling of a benzyl-protected 1,3-dibromophenyl precursor with 3-ethynlypyridine, followed by basic deprotection of the benzyl ester to afford the free acid, forming the highly conjugated bispyridyl ligand scaffold in good yields (Scheme S1). A polyethylene glycol \(exo\)-functionalized ligand \((L_3)\) was also synthesized. In this case, a ligand bearing a central phenol aromatic ring was synthesized via Sonogashira cross-coupling, which was then coupled to 1-chloro-2-(2-methoxyethoxy) ethane under basic conditions to achieve ligand \(L_3\) (Scheme S2). In addition, ligands \(L_4\) and \(L_5\), featuring water-soluble thio-\(\beta\)-D-glucose moieties, were synthesized for the first time. This was achieved by first synthesizing an alkyl bromide \(exo\)-functionalized ligand via classic Appel reaction using triphenylphosphine, carbon tetrabromide, and 3,5-bis(3-ethynlypyridine) benzylalcohol; the latter having been synthesized via Sonogashira cross-coupling (Scheme S3). The 1-thio-\(\beta\)-D-glucose tetaacetate group was then conjugated under basic conditions following a \(\text{Sn2} \) type reaction to afford the ligand \(L_4\). Ligand \(L_5\) was achieved by deacetylation of \(L_4\) using Amberlite-IRA 401 (\(\text{OH}^-\)) ion exchange beads, which were stirred together at room temperature overnight, following previously reported procedures (Lewis et al., 2014). Finally, the ligands \(L_6\) and \(L_7\) were synthesized using standard CuAAC “Click” reaction conditions (Scheme S4) following similar procedures reported for analogous tris-pyridyl ligand scaffolds (Lewis et al., 2013). Noteworthy, the latter were reported to be soluble in water, and therefore, offered a logical starting point for functionalization of the herein selected 3,5-bis(3-ethynlypyridine) phenyl ligands. All the ligands were characterized by NMR (\(\text{\(\text{H}, \text{\(\text{C} \)C}\)}\)) and HR-ESI-MS. A summary of the synthesized ligands and cages can be found in Scheme S5.

The solubility of the ligands in water was assessed by overnight sonication in \(D_2O\) (10 mg/mL, 20°C), before filtration (pore size 450 µm). The filtrate was analyzed by \(\text{\(\text{H} \)NMR\)} to determine if detectable amounts of ligand were present. Unfortunately, the \(exo\)-functionalized bispyridyl ligand scaffolds were found to be scarcely soluble, and were all undetectable in \(D_2O\) solution, except for the carboxylic acid functionalized ligands \((L_1 = 10 \text{ mg/mL}, \ L_2 > 100 \text{ mg/mL})\). The latter could be analyzed by \(\text{\(\text{H} \)NMR\)} in \(D_2O\) (data not shown).

Afterwards, \([\text{Pd}_2\text{L}_4]^{4+}\) cage formation was achieved via self-assembly by reacting two equivalents of Pd(II) nitrate pentahydrate with four equivalents of the functionalized ligand \((L_1-L_7)\) in DMSO at room temperature, to form the corresponding homoleptic metallacages \((C1-C7)\) (Scheme 1). The products were analyzed by NMR (\(\text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}) and IR. Following previously reported examples (Schmidt et al., 2016c), quantitative cage formation was monitored by \(\text{\(\text{H} \)NMR\)}, whereby the peaks corresponding to the protons alpha to the coordinating nitrogen of the terminal pyridine rings of the ligand undergo a large downfield shift, attributable to the withdrawal of electron density from the aromatic system following coordination to palladium (added in stoichiometric amounts). A representative \(\text{\(\text{H} \)NMR\)} spectra for cage \(C2\) formation is shown in Figure S4.

In order to assess whether the synthesized metallacages were soluble in water, the products were suspended in \(D_2O\) (8 mg per mL) and sonicated at 37°C for 24 h. The fine suspension was then filtered, and the filtrate was analyzed by \(\text{\(\text{H} \)NMR\)} (\(\text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}, \text{\(\text{H}, \text{\(\text{C} \)C}\)}) and IR. Despite the tetra-cationic nature of the metallacages, and the incorporation of solubilizing entities to the ligand scaffold, none of the metallacages \((C1-C7)\) were soluble in \(D_2O\). In fact, the filtrate solution was colorless and no trace of a proton signal could be found in the \(\text{\(\text{H} \)NMR\)} spectra.

As a second attempt to water solubilization, and taking inspiration from previous work by Altmann and Pöthig (2016) on the synthesis of water soluble Ag(I) and Au(I) pillarplexes,
we synthesized new cages featuring acetate as the counterion by the use of palladium(II) acetate as the precursor in the self-assembly. However, this attempt produced an insoluble residue that could not be dissolved in either organic solvents or water.

Concerning cisplatin encapsulation, in the case of previously reported \([\text{Pd}_2\text{L}_4]^{2+}\) cages (\(L = (2,6\text{-bis(pyrindin-3-ylethynyl)pyridine})\)) it was hypothesized that their central cavities are lined with four hydrogen bond accepting pyridine units which enable the encapsulation of two cisplatin molecules within the metallosupramolecular architecture through hydrogen bonding interactions between the cage and the amine ligands of the cisplatin guest (Lewis et al., 2012). This idea has been supported by NMR spectroscopy studies (Lewis et al., 2012), where chemical shifts of hydrogens in the trispyridyl ligands were observed upon cisplatin encapsulation. Control experiments confirm the importance of the hydrogen bonding interaction between the cage and the amine ligands of the cisplatin guest. It was also observed that upon addition of small amounts of D$_2$O to a solution of the cisplatin-cage host-guest adduct in acetonitrile, the H$_a$ and H$_b$ proton signals of the cage sharpen and shift upfield again, indicating evacuation of cisplatin from the cavity (Lewis et al., 2012).

In this context, information on the encapsulation process of cisplatin within the more hydrophobic cavity of trispyridyl-ligand \(\text{Pd}_2\text{L}_4\) metallacages (Lewis et al., 2012, 2014), needs to be achieved still. Thus, we investigated if the encapsulation of the anticancer drug cisplatin could occur in the metallacages using $^1$H NMR spectroscopy. In order to avoid DMSO as the solvent, since it is known to exchange cisplatin's chlorido ligands, the experiment was carried out in DMF-$d_7$. Cage C1 \([\text{Pd}_2\text{L}_4](\text{NO}_3)_4\) was selected to study the encapsulation process due to its solubility in this solvent. Figure 1 shows the variation of the chemical shifts of selected cage protons upon cisplatin encapsulation. Specifically, we could clearly observe the chemical shifts of peaks H$_a$ and H$_b$: the peak of H$_a$, a cavity-facing proton, showed a small upfield shift of $\delta = -1.0$ Hz. The exo-facing proton, H$_b$, showed a downfield shift upon cisplatin encapsulation, in accordance with previously reported studies (Lewis et al., 2012). Unfortunately, the cavity facing peak H$_c$, likely to interact with the guest Pt(II) complex, was masked by the peak of H$_a$, and therefore, could not be analyzed.

It is worth mentioning that the observed smaller chemical shifts of the cavity facing proton H$_a$ with respect to those reported by Lewis et al. (2012) can be due to the lack of the H-bonding with the cisplatin guest (via N—H$_3$N). Moreover, it has been previously reported that NO$_3^-$ counterions are encapsulated within the cage architecture of trispyridyl \(\text{Pd}_2\text{L}_4\) metallacages featuring a nitrogen in endo-position, while BF$_4^-$ ions are not (Lewis and Crowley, 2014). Thus, we hypothesized
FIGURE 1 | Cisplatin encapsulation in a metallacage following displacement of nitrate ions from the cavity. Stacked spectra of cage C1 in DMF-d7 (top) and cage C1 after the addition of 2 equiv of cisplatin (bottom). Box: Zoom-in of signals for peaks H_a and H_b with chemical shifts of $\delta = -0.002$ ppm (upfield) and $+0.01$ ppm (downfield), respectively.
that the observed small chemical shift of H₄ following cisplatin encapsulation was due to the presence of NO₃⁻ ions inside the cage, which already induced chemical shifts of H₄ and H₅, and thus, acted as “mask” the effect of the cisplatin encapsulation process. In fact, upon evacuation of the anionic NO₃⁻ counterion, the “de-shielding” effect of the cavity facing protons from NO₃⁻ would be reduced as the neutral guest, cisplatin, occupies the metallacage cavity.

Afterwards, we also investigated if cisplatin would preferentially be encapsulated within our bispyridyl type Pd₂L₄ metallacages featuring a different counter ion. Therefore, an analogous metallacage was synthesized with BF₄⁻ as the counterion, C1.BF₄ and studied for its cisplatin encapsulation properties by ¹H NMR spectroscopy. Supporting Figure S10 shows the stacked ¹H NMR spectrum of C1.BF₄, alone or encapsulating cisplatin [C1.BF₄⊂(cisplatin)] in DMF-d₇. Indeed, both H₆ and H₇ show marked downfield shifts upon cisplatin encapsulation of 0.044 and 0.033 ppm, respectively. In addition, H₅ also shows a downfield shift of 0.03 ppm. Furthermore, the peaks of both H₄ and H₅ of C1 (before addition of cisplatin) are already shifted further downfield (Figure 1, ca. 0.095 and 0.001 ppm, respectively) when compared to H₆ and H₇ of C1.BF₄ (Figure S10) consistent with encapsulation of NO₃⁻.

To continue our investigation, the effect of the solvent polarity was hypothesized to be the driving force of the cisplatin encapsulation process. Thus, the latter was also tested using C1.BF₄ in MeCN-d₃, with reduced polarity with respect to DMF-d₇. However, upon addition of 2 eq. cisplatin to the MeCN-d₃ solution of C1.BF₄ (1 equation) a precipitate was formed, even after several hours of sonication. The suspension was filtered, and the resulting solution was analyzed by ¹H NMR spectroscopy. Unlike before, no indication of cisplatin encapsulation (data not shown). Instead, the large upfield shift of the peaks corresponding to the α-protons of the coordinating pyridyl is indicative of the ligands no longer coordinated to Pd(II) ions. Moreover, the NMR spectrum suggests that the structure of the ligand has also been altered.

Furthermore, in order to study the effects of the solvent on cisplatin encapsulation, we synthesized a derivative of cage C1.BF₄, but where the carboxylic function was protected by a benzyl group, namely cage C1Bn.BF₄. Thus, cisplatin encapsulation was followed by ¹H NMR in MeCN-d₃ and the results are presented in Figure S11. As it can be observed, no chemical shifts could be seen for any of the cage’s proton peaks even after 24 h incubation of cisplatin at room temperature. We could conclude that in acetonitrile, cisplatin encapsulation is not favored. In order to check that metallo-drug encapsulation was not prevented by the presence of the benzyl group on the cage, we also monitored the process once more in DMF-d₇. The results are depicted in Figure S12 and indicate that, in these conditions, cisplatin encapsulation occurs, as shown by the chemical shifts of H₄ (downfield, δ = +0.009 ppm), H₅ (downfield, δ = +0.021 ppm), and a small shift for the cavity-facing proton H₆ (upfield, δ = −0.002 ppm). The encapsulation of cisplatin in cage C1Bn.BF₄ was further studied in DMF-d₇ using ¹⁹⁵Pt NMR to see if a complementary shift could be observed for the guest cisplatin molecules. Indeed, an upfield shift of −5 ppm was observed upon addition of either 1 eq. or 2 eq. of cisplatin in the presence of metallacage C1Bn.BF₄ (Figure S13), supporting cisplatin encapsulation. The obtained results are in agreement with previously reported ones from our group on similar cage systems exo-functionalized with different type of ligands (Schmidt et al., 2016c), indicating that surface functionalization does not prevent the guest from entering the cavity.

The encapsulation of cisplatin in C1Bn.BF₄ was also studied by ³¹P DOSY NMR in DMF-d₇. Cisplatin alone shows a broad signal at 4.18 ppm, HNO₃ with a diffusion coefficient = −8.9 × 10⁻¹⁰ m²/s (Figure S14, red traces). Clear quenching of this signal was observed upon addition of 1 eq. of metallacage C1Bn.BF₄ (Figure S14, blue traces). The cisplatin peak reappears slightly upon addition of a second equivalent of cisplatin to C1Bn.BF₄⊂(cisplatin) (Figure S14, black traces), which may suggest that the metallacage cavity has been saturated to form C1Bn.BF₄⊂(cisplatin)₂ and residual cisplatin remains free in solution, or that upon addition of a second equivalent of cisplatin, a dynamic equilibrium occurs.

**CONCLUSIONS**

In conclusion, we report here on a series of exo-functionalized [Pd₂L₄]⁴⁺ metallacages featuring bispyridyl ligands. Despite the addition of hydrophilic groups to the ligands’ scaffold, or the use of different counter ions, the increase in water solubility of the resulting cages was not achieved. Thus, different strategies are necessary to address this problem, including the conjugation of the ligands to peptides. The latter approach has already proved to be successful (Han et al., 2017).

Furthermore, the encapsulation of the anticancer drug cisplatin in selected cages has been studied by NMR spectroscopy, and the obtained results show that if the solvent is of sufficient polarity, metallo-drug encapsulation can easily occur in the hydrophobic cavity of the cage despite the absence of hydrogen-bond accepting central pyridine units (Lewis and Crowley, 2014). Conversely, polar solvent molecules capable of forming hydrogen-bond networks (including DMF and water) are likely to prefer not to be encapsulated by hydrophobic cage cavities, as in our case, and will not compete with the cisplatin molecules. This hypothesis is corroborated by previous studies on M₄L₆ metallacages for which guest encapsulation in polar protic solvents, such as water, appears to be driven by initial desolvation of the guest with concomitant rearrangement of the hydrogen bond networks in solution, more than by host-guest interactions alone (Leung et al., 2008). In general, energetically favorable solvent rearrangement during guest desolvation also explains the ability of the supramolecular host to encapsulate neutral guests such as cisplatin, in spite of the lack of possible electrostatic attraction or H-bond interactions with the charged host, as in our case. Further studies are ongoing in our lab to validate this hypothesis.
AUTHOR CONTRIBUTIONS

AC and BW contributed conception and design of the study. BW, MW, and ST contributed to the design, synthesis, and characterization of the ligands, and of the palladium cages. TW contributed to the HR-ESI-MS analysis of the cages. BW and RJ performed the encapsulation studies by NMR spectroscopy. AC and BW wrote the first draft of the manuscript, while MW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2019.00068/full#supplementary-material

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