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Investigations of Metal-Coordinated Peptides as Supramolecular Synthons

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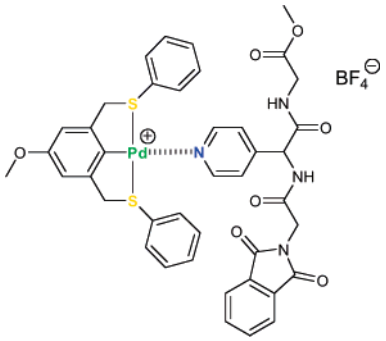

This article describes the synthesis and controlled assembly of four model biological-hybrid scaffolds via coordination of a metal complex to four new tripeptides. Each model tripeptide investigated has either a central pyridyl glycyl or a pyridyl alanyl residue between two terminally protected glycines. All tripeptides were coordinated to their complementary recognition unit, a *p*-methoxy SCS−Pd pincer complex. The assembly events were fully characterized and investigated by ^{1}H NMR, ES-MS, and isothermal titration calorimetry (ITC) to elucidate how the substitution and spatial distance of the pyridyl moiety to the peptide backbone affects the metal coordination. Using these characterization techniques, we have shown that the metal-coordination events in all cases are fast and quantitative and that the peptide backbones do not interfere with the self-assembly. The ITC analyses showed that the 4-pyridyl tripeptides are the tightest binding ligands toward the palladated pincer complexes with the alanyl derivative being the strongest overall, demonstrating the superiority of the 4-pyridyl peptides over their 3-pyridyl analogues. The measured association constants are comparable to other pincer−pyridine systems in DMSO suggesting that the controlled coordination of the metalated pincer/pyridine interaction is an interesting biological synthon and will allow for the future development of important noncovalent peptide-based hybrid materials.

Introduction

Organic supramolecular structures and assemblies hold great promise as building blocks for precision nanomaterials and structures that are otherwise conceptually inaccessible.[1−5] Through the use of controlled metal coordination at targeted positions in natural or synthetic peptides, one might be able to design well-defined persistent architectures,[6−8] thereby creating self-assembled biologically relevant and unique materials. Herein, we present as a step toward this goal the coordination of peptides with metalated pincer complexes, by embedding complementary recognition units in the peptide side-chain.

In biomacromolecules, metal coordination plays an essential role in many of life's processes.[9−11] And in the burgeoning field of supramolecular chemistry, metal coordination already has a

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vital role in the assembly of purely synthetic systems,[12−14] creating geometric and highly controlled architectures[12−20] or functional polymeric materials.[21−32] However, its use in the assembly of biological suprastructures, specifically with peptides, is not nearly as developed. The early use of metal coordination to control the design and folding of natural peptides was reported in 1990 by Ghadiri and co-workers[33,34] and Degrado and co-workers.[35] Ghadiri coaxed linear peptides to form helices by cross-linking the peptides with transition-metal ions via side-chain coordination of two natural residues. Degrado used coordination of histidine residues to Zn^{2+} to fold a peptide into a structural approximation of the enzyme carbonic anhydrase.[36] Sasaki and co-workers were the first to use unnatural ligands to self-assemble triple-helix bundles by chelating Fe^{2+} with bipyridines covalently attached to the peptides' N-termini.[37,38]

The synthesis of peptides with unnatural residue side-chains was the next step toward greater complexity and control in metal-coordinating peptides. Novel synthetic residues preserve the peptide backbone and can be redefined ad infinitum creating diversity within supramolecular systems.[39] Toward this goal, Hopkins and co-workers synthesized a peptide with two identical unnatural residues containing a diiminoacetic acid side-chain that was able to induce helix nucleation.[40]

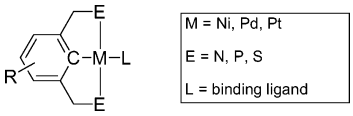

FIGURE 1. Pincer metallacycle: E is a neutral two-electron donor; R is a tethering group.

After Hopkin's seminal work, there were many reports using peptides with unnatural amino acids with metal coordination to stabilize or create defined nanostructures such as helixes,[41,42] loops,[43] cycles,[39] and other scaffolds.[44−46] Our report is the first study of peptides containing side-chain recognition units coordinated to a metalated pincer complex. We envision these peptides as models. Once fully understood, they can be situated into a cyclic peptide framework and coordinated with multifunctional/faceted metalated receptors into larger networks and structures.

Metalated pincer complexes (Figure 1) have received considerable attention within the field of supramolecular chemistry.[47] This chemo-activated, single-site metal-coordination step has been shown to be fast, quantitative, and chemo reversible in a variety of solvents.[16,41,47−50] Incorporation of one of its ligands into the side-chain of an unnatural peptide would open a simple and direct avenue to new peptide-supramolecular structures, making the metalated pincer complex an ideal candidate for achieving our goals.

The pincer ligand is a tridentate ligand that after metalation, with a Pd or Pt, results in a four-coordinate square planar metal complex.[51−53] The three chelation sites most often have a central carbon flanked by two neutral donor atoms (E) giving it an ECE nomenclature.[47] In this investigation, we used sulfur as the donors and Pd as the metal center, i.e., an SCS−Pd pincer complex. The fourth site is normally occupied by a halide that can be abstracted quantitatively by a suitable silver salt.[54] This is the chemo trigger, which precipitates a silver halide leaving an ionic palladium species with an open coordination site that

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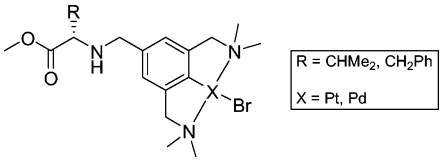

FIGURE 2. Pincer amino acids synthesized by van Koten.

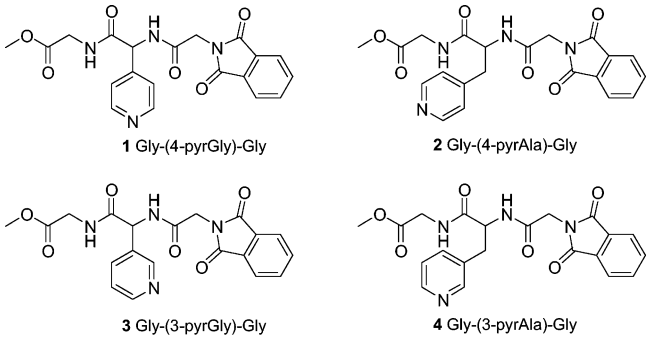

FIGURE 3. Four model tripeptides containing an unnatural pyridyl glycine or pyridyl alanine as the central residue.

can be quantitatively complexed by a donor ligand (vida supra) forming a strong directional metal−ligand bond.[48,55]

Currently, the only reported use of a metalated pincer complex with a peptide or an amino acid was described by van Koten and co-workers.[56,57] In this case, NCN−Pt pincer and Pd pincer complexes were covalently attached to the N-terminus of an amino acid (Figure 2) and then a peptide chain. However, in their articles, the authors did not describe this system as an assembly unit. Nevertheless, we evaluated their elegant approach as a possible strategy toward the controlled noncovalent synthesis of peptide-based materials. Unfortunately, the functionalization at the N-terminus of the peptide clearly limits the use of this design as a general synthon in metal-coordination-based peptidal architectures.

For our approach, we chose a pyridyl side-chain residue as a selective recognition unit for the metal coordination to the SCS−Pd pincer complex. A pyridyl moiety was chosen as the functional side-chain because (a) there are already a handful of reports using nitrogenous heteroaromatic side-chains as receptors for self-assembly in this area of supramolecular chemistry[41−46] and (b) the use of pyridine as a ligand for metalated pincer complexes has been established over the past decade.[15,25,26,32,47,48,58,59]

In this initial contribution, we synthesized and investigated four tripeptides as supramolecular synthons **1**−**4** (Figure 3), each with the unnatural pyridyl side-chain residue in the center being flanked by two terminally protected glycine residues.

Each of these four peptides has a different substitution pattern and spacing of the central pyridine side-chain from the peptide backbone. These variations are expected to lead to different coordination geometries and strengths. We studied the metal-

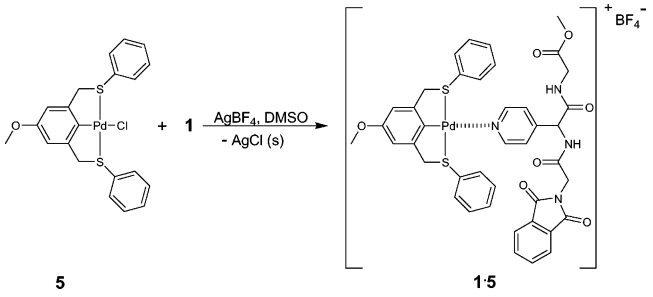

FIGURE 4. Activation and coordination of *p*-methoxy SCS−Pd pincer complex **5** to tripeptide **1** in DMSO.

coordination capabilities of tripeptides **1**−**4** with *p*-methoxy SCS−Pd pincer complex **5** (Figure 4) in DMSO because a self-assembled biomaterial should have a strong noncovalent interaction that is fast and quantitative in a polar environment. The behavior of each unique coordination event was characterized by ^{1}H NMR spectroscopy, isothermal titration calorimetry (ITC), and ES-mass spectroscopy to determine which substitution pattern and spacing distance is ideal for these model synthons, to establish association constants (K_a) and to ascertain any influences of the peptide backbone on this coordination. Ultimately this detailed analysis will allow us to develop novel cyclic peptide supramolecular synthons coordinated with metalated pincer complexes giving architecturally complex biomaterials and scaffolds.

Results and Discussion

Metalated pincer complexes are known to coordinate to other nitrogen-containing heteroaromatic units such as pyrazine.[32] Therefore, if histidine with its imidazole side-chain would coordinate quantitatively to metalated pincer complexes, histidyl peptides or related derivatives would be the simplest and most direct route toward our goal. Therefore, we first examined the coordination of imidazole and methyl imidazole with a SCS−Pd pincer complex as a preliminary study. However, the ^{1}H NMR spectra of the metal coordination of both imidazole and methyl imidazole to metalated pincer complexes showed no clear or strong coordination pattern indicating that no clean or controlled single metal coordination event took place.

Peptide Synthesis. On the basis of the unsatisfactory imidazole/methyl imidazole−Pd pincer study, we used pyridine, which is the most common ligand for metalated pincer complexes,[47] in our design. The four distinct tripeptides (**1**−**4**) were made with a pyridyl glycine or alanine amino acid via solution-phase peptide synthesis using two different strategies (Scheme 1 shows the synthesis of the pyridyl alanines and Scheme 3 shows the synthesis of the pyridyl glycines). The C-termini of all tripeptides were protected as methyl esters, and the N-termini were protected with a phthaloyl group. After purification by RP-HPLC, these tripeptides crystallized easily from a CH_3CN/water mixture (**1** was the exception). The crystal structures of **2**−**4** can be found in the Supporting Information.

Pyridyl Alanine Tripeptide Synthesis. Protected versions of the 3- and 4-pyridyl alanine amino acids are commercially available, and tripeptides **2** and **4** were synthesized using conventional peptide coupling techniques. A nonfunctional isosteric phenylalanine tripeptide **6** was also synthesized using this route with comparable yields (Scheme 2).

The syntheses of the Boc-protected dipeptides **7**−**9** were carried out using dicyclohexyl carbodiimide (DCC) as the

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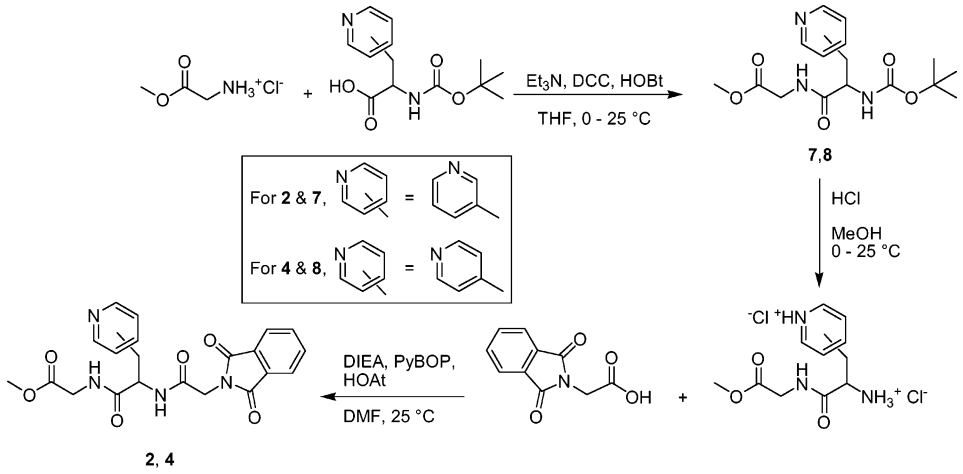
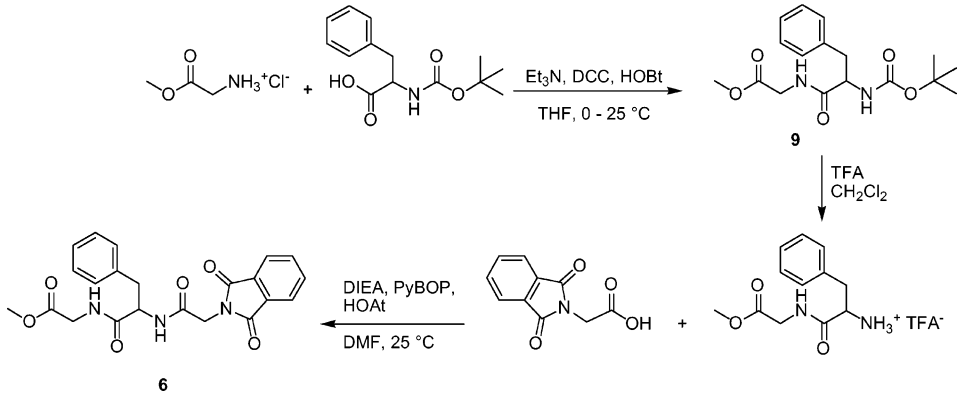
SCHEME 1. Synthesis of Tripeptides 2 and 4



SCHEME 2. Synthesis of Nonfunctional Isosteric Tripeptide 6



condensing agent and the nucleophilic additive 1-hydroxybenzotriazole (HOBt) in THF with yields of 88%, 95%, and 90% for **7**, **8**, and **9**, respectively. The Boc group of dipeptide **9** was removed using a 1:2 TFA/CH_2Cl_2 solution. However, Boc deprotection of dipeptides **7** and **8** with TFA led to trifluoramide terminated dipeptides under a variety of coupling conditions. Therefore, these Boc groups were removed with a saturated methanolic solution of HCl. The best results for the tripeptidation coupling step were realized with benzotriazole-1-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate (PyBOP) as the condensing agent and the nucleophilic additive 1-hydroxy-7-azabenzotriazole (HOAt), giving overall yields of 78%, 64%, and 87% for **2**, **4**, and **6**, respectively.

Pyridyl Glycine Tripeptide Synthesis. The synthetic route toward glycyl derivative **1** was not as straightforward as that for the alanyl derivatives. Not commercially available, the 4-pyridyl glycine residue was first synthesized but never isolated as the carboxylic acid because of an inherent instability, which led to spontaneous decarboxylation upon acidification of the lithium carboxylate salt to isolate the pyridinium amino acid residue. This exacerbated the synthesis of the pyridyl glycyl amino acid of **1**; therefore, a different synthesis in comparison to that for **2** and **4** was developed that went directly to the dipeptide. This was done using a modified Strecker synthesis developed by Ugi and further expanded by Kunz (Scheme 3).[60−66]

The synthetic protocol used a protected amino sugar as an auxiliary, forming an imine with the appropriate pyridine carboxaldehyde precursor, that is attacked by the isonitrile to give the protected dipeptides **10** and **11** with yields greater than 90%. The N-terminus protections were removed with a two-part, one-pot methanolic/aqueous HCl deprotection. After re-esterification, the HCl salts of **10** and **11** were then converted to tripeptides **1** and **3** using the same tripeptidation step as the alanyl derivatives, with overall yields of 86% and 70% for **1** and **3**, respectively.

***p*-Methoxy SCS−Pd Pincer Synthesis.** The synthesis of the palladated pincer complex **5** started with the methylation of **12** to **13** using iodomethane, which was then palladated to yield **5** following standard literature procedures (Scheme 4) with an overall yield of 92%.[58,67,68] Palladated pincer complex **5** could be further recrystallized from $CHCl_3$ as large yellow crystals.

Coordination. Peptides **1**−**4** were synthesized to fully characterize and understand the coordination pattern and association strength of each new peptide ligand. The limited solubility of all four peptides and their coordinated complexes in less-polar organic solvents ($CHCl_3$ and CH_2Cl_2) that are traditionally used to assemble metalated pincer−ligand com-

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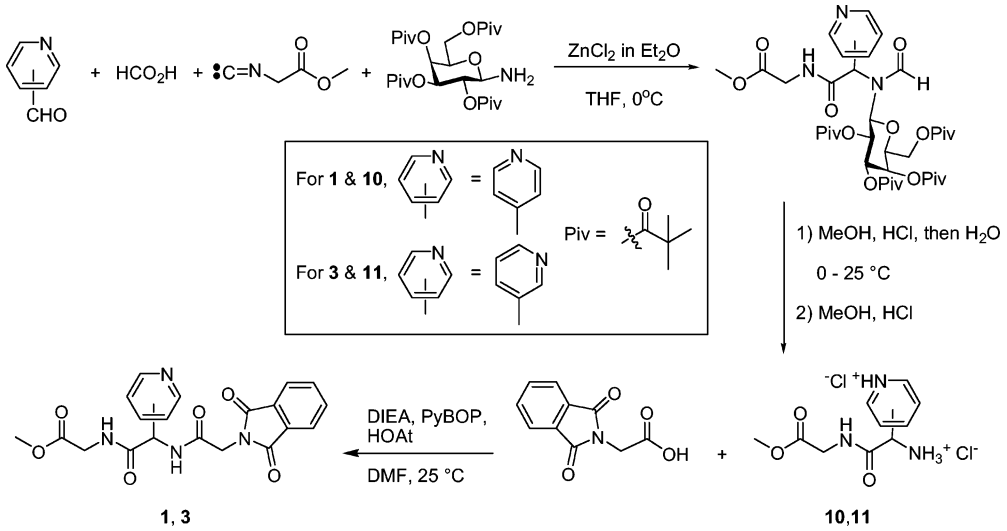
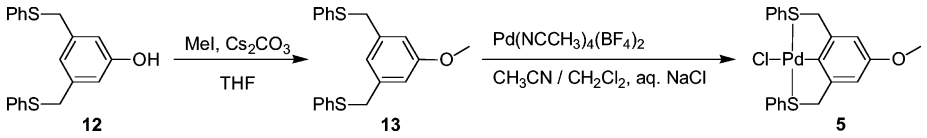
SCHEME 3. Synthesis of Tripeptides 1 and 3 Using a Modified Strecker Synthesis



SCHEME 4. Synthesis of *p*-Methoxy SCS−Pd Pincer 5



plexes was a challenge. Because the overall goal of this project is to develop peptidal metal-coordinated self-assembly in highly polar solvents, all of our coordination studies were carried out in DMSO. Several contributions of metalated pincer complexations in polar solvents such as MeOH, H_2O, and DMSO have been reported in the literature.[18,48−50,69] A significantly weaker binding in DMSO was expected vs assemblies in $CHCl_3$ and CH_2Cl_2 because of polarity differences as well as its competitive coordination which leads to solvent-assisted dissociations and faster exchange rates.

NMR Characterization of the Coordination. For all NMR experiments, peptides **1**−**4** were dissolved in d_6-DMSO in an NMR tube and **5** was titrated into the DMSO solution until a 1:1 molar ratio was established. Then, 1 equiv of $AgBF_4$ in d_6-DMSO was added that led to an instant precipitation of AgCl. This was the first evidence of coordination. The salt was then filtered off, and each coordinated species was analyzed.

Pincer ligand−metal complexes are traditionally characterized by ^{1}H NMR spectroscopy.[48,70] The protons in resonance with or near the site of coordination generally show characteristic shifts in the ^{1}H NMR spectrum of the coordinated species vs its uncoordinated counterparts.[48] A 1:1 molar solution of the tripeptides and Pd pincer complex **5** in d_6-DMSO showed quantitative shifts in the signals of interest (Figure 5 shows as an example the coordination of 4-pyridyl alanyl tripeptide **2** with Pd pincer complex **5**).

The observed shifts for the metal coordination of all four peptides with **5** are tabulated in Tables 1 and 2 and assigned in Figures 6 and 7. These characteristic shifts in d_6-DMSO are not as dramatic as shifts seen in less-coordinating organic solvents.[19,26,70] Because DMSO can facilitate the displacement and exchange of coordinated−uncoordinated pyridines via a solvolysis pathway, this fast equilibrium is also responsible for the signal broadening.[48] All observed shifts are caused by both changes in the electronics of each unit of the supramolecular system after coordination and overlap of protons in new ring currents as the two coordinated molecules come into close proximity.[48] These effects can counteract and interfere with each other, leading to the erratic shifts in each system and complicating the determination of these weaker binding constants using ^{1}H NMR spectroscopy.

Because this is the first peptide−pincer complex coordination study, we wanted to clearly establish that all the observed shifts are due to a 1:1 well-defined coordination. In all coordination experiments, subtle shifts of the amide protons between 8.5 and 9.0 ppm occurred. These shifts could be innocuously caused by the overall change in the electronics of the molecule through the coordination of the pincer solely at the pyridine or through a perturbation of the hydrogen-bonding network of these protons via coordination of the amide nitrogens with a small percentage of residual silver salts still in solution. Alternatively, an undesirable complexation of the amide protons to the activated metalated pincer complex may be responsible for these shifts resulting in an ill-defined system.

To determine the nature of the amide proton shifts, we characterized a 1:1 mixture of $AgBF_4$ and peptide **2** using ^{1}H NMR spectroscopy. The^{1}H NMR spectrum showed very slight shifts (less than 0.1 ppm) of the β-pyridine protons and the amide protons, implying that some interaction of backbone nitrogens with excess residual silver ions does occur.

To probe if a noncovalent interaction of the pincer complex with the amide backbone was also taking place, we prepared a 1:1 mixture of nonfunctional isosteric peptide **6** and complex **5** in d_6-DMSO and acquired the ^{1}H NMR spectrum of the solution before and after the addition of 1 equiv of $AgBF_4$. There was no change in any of the peptide signals after addition of the silver salt. Therefore, the shifts observed in the backbone amide protons are due to a change in the overall electronics after

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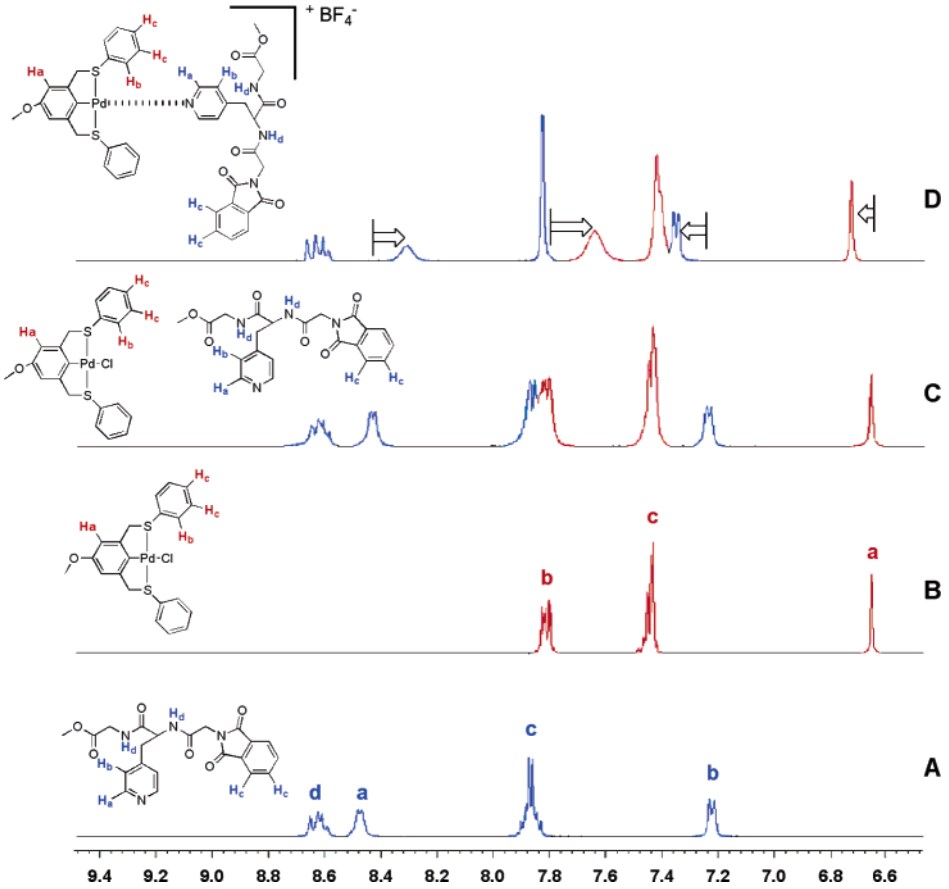

FIGURE 5. Stacked ^{1}H NMR spectra in d_6-DMSO depicting metal coordination of **5** to **2**, with the arrows in D pointing toward the diagnostic shifts: (A) peptide **2**, 0.01 M; (B) pincer complex **5**, 0.01 M; (C) 1:1 mixture of **2** and **5**, 0.006 M; (D) 1:1 mixture of **2** and **5** after the addition of 1 equiv of $AgBF_{4(s)}$, 0.006 M.

TABLE 1. ^{1}H NMR Shifts in ppm of Relevant Protons on 4-Pyridyl Peptides (1 and 2) and 5 before and after Metal Coordination, with a Final Concentration of 0.006 M of Each Component in d_6-DMSO

peptide **1** proton no.	ppm before self-assembly	ppm after self-assembly	peptide **2** proton no.	ppm before self-assembly	ppm after self-assembly
H^{α}	8.56	8.48	H^{α}	8.45	8.38
H^{β}	7.45	7.52	H^{β}	7.31	7.35
H^1	7.43	7.44	H^1	7.43	7.39
H^2	7.82	7.65	H^2	7.82	7.65
H^3	6.65	6.72	H^3	6.65	6.72

TABLE 2. ^{1}H NMR Shifts in ppm of Relevant Protons on 3-Pyridyl Peptides (3 and 4) and 5 before and after Metal Coordination, with a Final Concentration of 0.006 M of Each Component in d_6-DMSO

peptide **3** proton no.	ppm before self-assembly	ppm after self-assembly	peptide **4** proton no.	ppm before self-assembly	ppm after self-assembly
H^{α}	8.50	8.36	H^{α}	8.43	8.34
H^{β}	8.62	8.63	H^{β}	8.43	8.34
H^{γ}	7.79	7.90	H^{γ}	7.64	7.73
H^{δ}	7.40	7.47	H^{δ}	7.30	7.41
H^1	7.43	7.44	H^1	7.43	7.41
H^2	7.82	7.72	H^2	7.82	7.73
H^3	6.65	6.71	H^3	6.65	6.65

coordination and to a lesser extent to secondary interactions of the amide nitrogens with residual silver ions. These two results clearly prove that no coordination took place between the amide backbone and complex **5** and that complexation occurs only at the nitrogen pyridine in a highly controlled fashion.

Characterization of the Peptide−Pd Pincer Complex by Mass Spectrometry. In the literature, it has been reported that complexed pincer systems can be analyzed by mass spectrometry (MS) using several different ionization techniques (FAB, MALDI-TOF, ESI).[19,20,50,55] We found that electrospray (ES) ionization in a neutral medium of a 1:1 mixture of water and methanol was mild enough to permit detection of the complexed species. The spectra for the complexes **1**·**5** and **3**·**5** showed a signal at 867.1 corresponding to the molecular ion peak of [(**1**·**5** or **3**·**5**) − BF_4]$^+$. Other significant signals present in the spectrum are at 411.1 (**1**·H^+ or **3**·H^+) and 457.0 for [**5** − Cl]$^+$

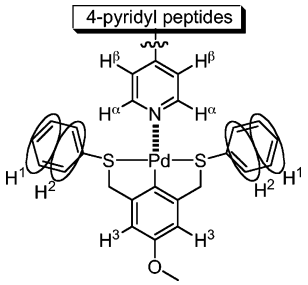

FIGURE 6. Proton assignment for 4-pyridyl peptides (**1** and **2**) coordinated to **5**.

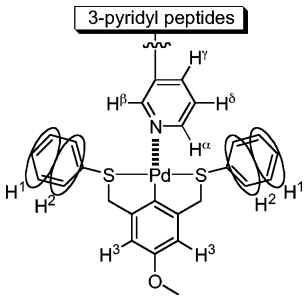

FIGURE 7. Proton assignment for 3-pyridyl peptides (**3** and **4**) coordinated to **5**.

corresponding to the peptide and pincer building blocks, respectively. These signals are most likely due to disassembly events during the ionization in the mass spectrometer. Related signals in the ES-MS spectra of **2·5** and **4·5** again displayed the characteristic signal at 881.4 corresponding to the molecular ion signal of the complexed species $[(\mathbf{2\cdot5}$ or $\mathbf{4\cdot5}) - BF_4]^+$ and the disassembled signals at 425.2 ($\mathbf{2}\cdot H^+$ or $\mathbf{4}\cdot H^+$) and 457.2 for $[\mathbf{5} - Cl]^+$.

Characterization of the Bond Strength Using Isothermal Titration Calorimetry (ITC). After affirming by ^{1}H NMR and ES-MS that complexation occurred, we investigated the bond strength of each coordination event using ITC. This was done to identify the optimal synthon, i.e., the strongest building block for envisaged biological cyclic networks. ITC, a technique widely used by biochemists, is quickly becoming routine in supramolecular chemistry[71] because of its ability to directly measure the enthalpy of binding to provide a complete thermodynamic characterization.[72,73] In our ITC experimental set up, we titrated each peptide into a DMSO solution of the acetonitrile-coordinated Pd pincer complex (Figure 8), and all experiments were run in triplicate to validate all reported K_a values.

Activation of **5** and subsequent coordination with CH_3CN to **14** was carried out prior to the ITC experiments to fully remove any AgCl precipitates, which could foul the ITC sample cell. CH_3CN was complexed to the palladium center to occupy this open coordination site. Our naked pincer complex appears to not be adequately coordinated by the DMSO solvent as a reasonable titration curve could not be generated from it,[69] possibly because of a rapid decomposition. It is well documented in the literature that nitriles are instantaneously replaced as ligands on Pd pincer complexes by pyridines or phosphines.[47,74] Therefore, the nitrile complexed onto the palladium centers can be viewed as a labile ligand (or protecting group) that is replaced by the tripeptides quantitatively upon addition.

The pincer metal coordination is regarded as a strong noncovalent interaction and is essentially irreversible in low polarity solvents (ITC measurements of pyridines coordinating to pincer complexes in $CHCl_3$ were above the upper limits of our instrument, K_a values $>10^9$), but because of the highly polar and coordinating nature of DMSO, the K_a values are known to be significantly lower.[48,49] We detected the same trend for the coordination of all four tripeptides with **5**. In all cases, highly reproducible hyperbolic titration curves characteristic of a weaker binding event were observed (Figure 9).[75]

The 4-pyridyl systems have K_a values >1200 M^{-1}, whereas the 3-pyridyl systems have K_a values <800 M^{-1} (Table 3). These K_a values fall between those of 4-picoline and pyridine, respectively, showing that the peptide backbone does not interfere with the coordination of the pyridyl moiety to the Pd pincer complex.

Although crystal structures of the coordinated complexes could reveal the nature of the coordination geometries and possibly shed light on each K_a value, none of the tripeptide complexes formed crystals suitable for characterization via single-crystal crystallography. Attempts can be made to explain the different K_a values through a combination of sterics and electronics. The ITC data show the superiority of the 4-pyridyl peptides on the basis of the available X-ray structures of uncoordinated tripeptides **2**−**4**. It can be inferred that the nitrogen of the pyridyl moiety is presumably more sterically accessible in the para-substituted 4-pyridyl peptides (**1** and **2**) vs the meta-substituted 3-pyridyl peptides (**3** and **4**), accounting for part of the difference in K_a values. The tighter binding of all four tripeptides vs pyridine can be rationalized entropically with all tripeptides having fewer degrees of freedom compared to pyridine and through the activation via electron donation of the methylene or methine groups adjacent to each of the pyridines in the peptide, which should activate them and ultimately increase their association constants. The stronger association of the alanyl derivatives vs their glycyl homologues can most likely be attributed to this same electronic activation, which is greater in the alanyl derivatives because of the greater electronic decoupling of the pyridine ring from the peptide backbone via the methylene spacer. Similar observations have been reported previously by Reinhoudt[48] and van Koten[76] establishing a Hammett-like relationship of binding strength to electron donation ability of the para substituent on multiple pyridine ligands. The K_a values of the 4-pyridyl peptides, **1** and **2**, are comparable to the coordination events in other supramolecular systems[77−79] and will make excellent additions to the peptidal-supramolecular repertoire.

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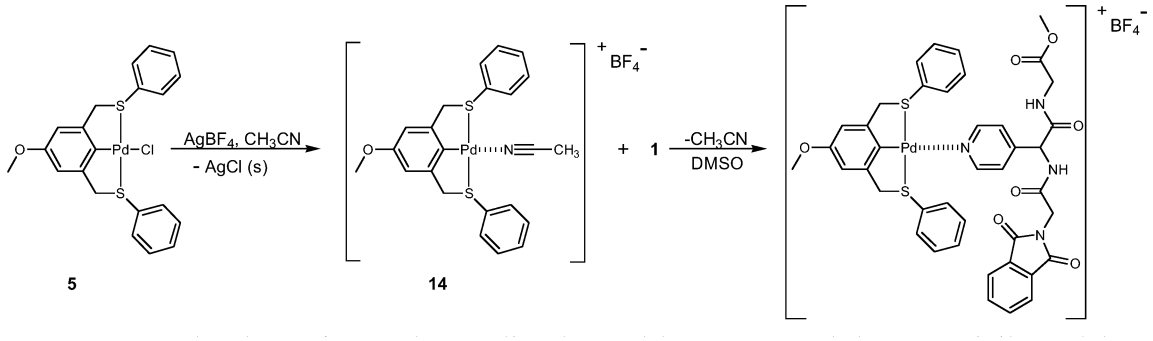

FIGURE 8. Activation of **5** and coordination with CH_3CN, giving **14**, followed by displacement of CH_3CN by **1**.

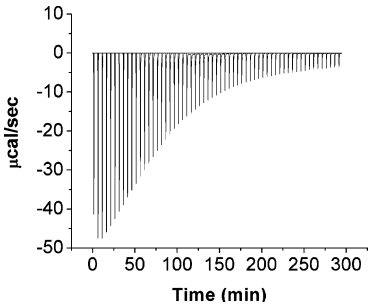

FIGURE 9. ITC curve of tripeptide **2** displacing a CH_3CN ligand from activated pincer complex **14**.

TABLE 3. ITC K_a Values of Tripeptides 1−4, Pyridine, and 4-Picoline Displacing CH_3CN from Activated Pincer Complex 14

complex	K_a
1−**5**	1190 ± 108
2−**5**	1580 ± 194
3−**5**	521 ± 54
4−**5**	757 ± 87
pyridine−**5**	382 ± 91
4-picoline−**5**	1730 ± 365

Conclusion

We have synthesized four new unnatural tripeptides (**1**−**4**) containing a central residue with a pyridyl side-chain. The complexation of each tripeptide with a *p*-methoxy-terminated SCS−Pd pincer complex (**5**) was investigated in detail. The metal coordination was characterized and evaluated by ^{1}H NMR spectroscopy, ES-MS, and ITC. Each spectroscopic method confirmed that coordination of each tripeptide to the Pd pincer complex has occurred quantitatively without any competitive coordination of the peptide backbone heteroatoms. The ITC analyses also showed that the 4-pyridyl tripeptides, **1** and **2**, are the tightest binding ligands toward **5**. Alanyl derivative **2** was the strongest overall demonstrating the superiority of the 4-pyridyl peptides over their 3-pyridyl analogues. Finally, the measured K_a values are comparable to other pincer−pyridine systems in DMSO[49,69] suggesting that the controlled coordination of the metalated pincer−pyridine interaction is an interesting biological synthon, and with the use of multifaceted 3-D receptors, these peptide ligands can be used to assemble new and interesting biological scaffolds.

This is the first study that describes the controlled coordination of a palladated pincer complex with unnatural peptide sequences as its complementary ligand. Our next step in this area will be to incorporate the unnatural residue ligands into cyclic peptides and self-assemble them with bifunctional metalated pincer complexes creating predetermined architectures and scaffolds such as those seen in purely synthetic supramolecular systems.[12−14,16,17,19,20]

Experimental Section

4-(1-Ammonio-2-(2-methoxy-2-oxoethylamino)-2-oxoethyl)-pyridinium Chloride, 10. $ZnCl_2$ in Et_2O (11.7 mL, 11.7 mmol) was added to a 0 °C solution of 2,3,4,6-tetra-*O*-pivaloyl-*β*-D-galactopyranosylamine (3.00 g, 5.8 mmol), 4-pyridinecarboxaldehyde (0.56 mL, 5.8 mmol), formic acid (0.24 mL, 6.4 mmol), and methyl isocyanoacetate (0.56 mL, 6.1 mmol) in anhydrous THF (25 mL). After 30 min, the reaction mixture was allowed to warm to 25 °C and monitored by TLC which showed complete consumption of the aldehyde after 12 h. The solvent was removed under reduced pressure, and the crude mixture was redissolved in EtOAc forming an orange solution, which was washed with a 1 N aqueous $KHSO_4$ solution (2 ×) removing the orange color and leaving a pale yellow solution. The organic layer was then washed (3 ×) with a saturated aqueous $NaHCO_3$ solution precipitating out a white solid. Residual precipitate was filtered off of the organic layer, and the solvent was removed under reduced pressure giving a yellow solid, which was further purified by flash column chromatography (silica gel, eluant/EtOAc). The product was dried in vacuo to give a yellow foam (4 g, 92%). This sugar-formyl protected dipeptide intermediate was dissolved in MeOH (20 mL) cooled to 0 °C and saturated with dry HCl gas for 15 min and stirred at 0 °C for 1 h. The reaction mixture was allowed to warm to 25 °C and stirred for an additional 4 h to remove the formyl group. Water (20 mL) was then added to the methanolic reaction mixture and stirred for an additional 10 h. The MeOH was then removed under reduced pressure, and the aqueous reaction mixture was washed with CH_2Cl_2 until the organic washes were colorless. The water was removed under reduced pressure to give the hydrolyzed dipeptide, which was redissolved in MeOH (30 mL) and saturated with dry HCl gas for 15 min and allowed to stir for 3 h to reesterify the carboxylate terminus. Et_2O was added to the methanolic solution to precipitate out the crude dipeptide·HCl salt which was further purified by repeated triturations from a boiling Et_2O/MeOH solution and isolated as an off-white solid (1.46 g, 85%). ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 9.62 (t, 1H), 9.35 (bs, 3H), 8.98 (m, 2H), 8.15 (d, J = 6.7, 2H), 5.51 (bs, 1H), 3.91 (t, J = 6.7, 2H), 3.55 (s, 3H). ^{13}C NMR (d_6-DMSO, 75 MHz) δ: 169.2, 165.7, 150.8, 143.4, 125.6, 54.2, 51.9, 40.9. MS (FAB$^+$) [M + H$^+$] 224.1. HRMS (FAB$^+$) [M + H$^+$] calcd for $C_{10}H_{14}N_3O_3$, 224.10183; found, 224.10352.

Methyl 2-(2-(2-(1,3-Dioxoisoindolin-2-yl)acetamido)-2-(pyridin-4-yl)acetamido)acetate, 1. The HCl salt of the deprotected dipeptide **10** (1.50 g, 5.10 mmol) was dissolved in a solution of DIEA (1.7 mL, 10.2 mmol) in anhydrous DMF (20 mL). The reaction mixture instantly turned yellow, and the mixture was incubated for 10 min. In a separate flask, a solution of PyBOP (3.18 g, 6.1 mmol), phthaloyl glycine (1.26 g, 6.1 mmol), HOAt (0.83 g, 6.1 mmol), and DIEA (3.0 mL, 18.4 mmol) in anhydrous

DMF (10 mL) was allowed to incubate for 5 min and then added in one portion to the deprotected dipeptide **10** solution. The reaction turned red after 30 min, and a precipitate formed after 1 h. Stirring was continued for 12 h. The DMF was removed under reduced pressure. The mixture was taken up in CH_2Cl_2, and the insoluble material was filtered off, washed with CH_2Cl_2, and dried to give a white powder which was further purified by HPLC (water/CH_3CN gradient; C18 column) yielding a white solid (1.93 g, 93%). ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 9.62 (d, J = 8.2, 1H), 9.35 (t, J = 5.9, 1H), 8.98 (d, J = 5.4, 2H), 7.82−7.93 (m, 4H), 7.41 (d, J = 6.0, 2H), 5.51 (d, J = 8.2, 1H), 4.38 (s, 2H), 3.91 (dd, J = 3.0, 5.7, 2H), 3.55 (s, 3H). ^{13}C NMR (d_6-DMSO, 75 MHz) δ: 169.9, 168.9, 167.5, 166.1, 149.7, 147.0, 134.7, 131.6, 123.3, 122.0, 55.1, 51.8, 40.7, 39.9. MS (ESI$^+$) [M + H$^+$] 411.1311. HRMS (ESI$^+$) [M + H$^+$] calcd for $C_{20}H_{19}N_4O_6$, 411.1307; found, 411.1299. Elemental analysis calcd for $C_{20}H_{18}N_4O_6$: C, 58.53; H, 4.42; N, 13.65; O, 23.39. Found: C, 58.49; H, 4.58; N, 13.59; O, 23.34. The onset of decomposition between 252 and 255 °C occurred before a melting point.

Methyl 2-(2-(2-(1,3-Dioxoisoindolin-2-yl)acetamido)-3-(pyridin-3-yl)propanamido)acetate, 4. Boc-protected dipeptide **7** (2.02 g, 6.0 mmol) was dissolved in MeOH (50 mL) and saturated with HCl gas for 15 min with agitation while keeping the temperature constant at 25 °C. After complete HCl addition, the reaction was stirred for an additional 30 min. The solvent was then removed under reduced pressure, and the residue was dried in vacuo (complete deprotection was confirmed by ^{1}H NMR). The HCl salt of deprotected dipeptide **7** was dissolved in a solution of DIEA (2.0 mL, 12.3 mmol) in anhydrous DMF (20 mL), and the mixture was incubated for 10 min. In a separate flask, a solution of PyBOP (3.76 g, 7.2 mmol), phthaloyl glycine (1.48 g, 7.2 mmol), HOAt (0.98 g, 7.2 mmol), and DIEA (3.6 mL, 21.7 mmol) in anhydrous DMF (10 mL) was allowed to incubate for 5 min and then was added in one portion to the deprotected dipeptide **7** solution. The reaction became a red color after 30 min, and a precipitate formed after 1 h. Stirring was continued for 12 h, at which time the DMF was removed under reduced pressure, yielding a crude brown viscous oil. The oil was dissolved in a minimum amount of CH_2Cl_2 with a few drops of TFA and semipurified by flash column chromatography (silica gel, eluant; 94/5/1 EtOAc/MeOH/Et_3N). The product was dried in vacuo to give a yellow powder, which was further purified by HPLC (water/CH_3CN gradient; C18 column) yielding a white solid (1.65 g, 65%) that was recrystallized from a 95:5 CH_3CN/water mixture as thin white needles for X-ray structural analysis. ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 8.58−8.65 (m, 1H), 8.58−8.65 (m, 1H), 8.41−8.43 (m, 2H), 7.83−7.91 (m, 4H), 7.61−7.66 (m, 1H), 7.25−7.33 (m, 1H), 4.53−4.63 (m, 1H), 4.18 (dd, J = 10.8, 16.8, 2H), 3.9 (dd, J = 2.2, 3.5, 2H), 3.61 (s, 3H), 2.91 (dd, J = 4.7, 13.9, 1H), 2.79 (dd, J = 9.2, 13.9, 1H). ^{13}C NMR (d_6-DMSO, 75 MHz) δ: 171.0, 170.1, 167.4, 165.9, 149.9, 147.4, 137.0, 131.7, 131.6, 123.4, 123.2, 53.3, 51.8, 40.6. 39.8, 34.9. MS (ESI$^+$) [M + H$^+$] 425.1469. HRMS (ESI$^+$) [M + H$^+$] calcd for $C_{21}H_{21}N_4O_6$, 425.1459; found, 425.1456; Elemental analysis calcd for $C_{21}H_{20}N_4O_6$: C, 59.43; H, 4.75; N, 13.20; O, 22.62. Found: C, 59.02; H, 4.83; N, 13.25; O, 22.90. The onset of decomposition between 222 and 227 °C occurred before a melting point.

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Supporting Information Available: Materials and methods, general experimental details and detailed characterization data for compounds **2**, **3**, **5**−**9**, **11**, **13**, and **14**, crystal structure images of **2**−**5**, and crystallographic data as a cif file. This material is available free of charge via the Internet at http://pubs.acs.org.

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