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Cooperative Metal-Coordination and Ion Pairing in Tripeptide Recognition

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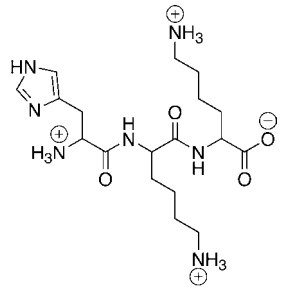
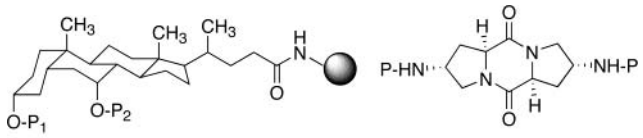
ABSTRACT



$K_a = 10^6\ M^{-1}$

The recognition of tripeptides by a metal-centered receptor consisting of a rigid backbone region and variable tripeptide arms is described. The studies showed that the receptor was selective for L-Xxx-L-Lys-L-Lys, with Xxx = His, Cys, and Met, giving association constants near 1.0 × 10^6 M^{-1}. Binding enhancement through cooperative interactions of the peptidic arms is demonstrated.

The selective recognition of short peptides and proteins by synthetic receptors is a current thrust in molecular recognition.[1] Short peptides such as hormones present challenging targets due to their inherent flexibility and structural diversity. Further challenges arise in developing receptors that function adequately in an aqueous environment.[2] Nevertheless, substantial gains have been made toward this, particularly through the utilization of combinatorial chemistry.[3] This technique allows the facile synthesis of many receptors in contrast to the more challenging designed receptors.[4] Still's steroidal enkephalin receptor **1** that has two variable peptide arms attached to a steroidal core[5] is a good example. Multiple-armed receptors with more rigid backbones and "tweezer-like" receptors such as Wennemer's diketopiperazine host[6] **2** have proven to be advantageous.



1 **2**

P_n = peptide arms

Recently, we showed that tweezer-like receptor **3** exhibits good selectivity for aspartic acid.[7] The metal center preorganizes the receptor for binding by converging the guanidinium groups together.[8] The binding is primarily due to a

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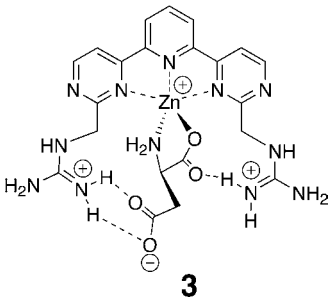
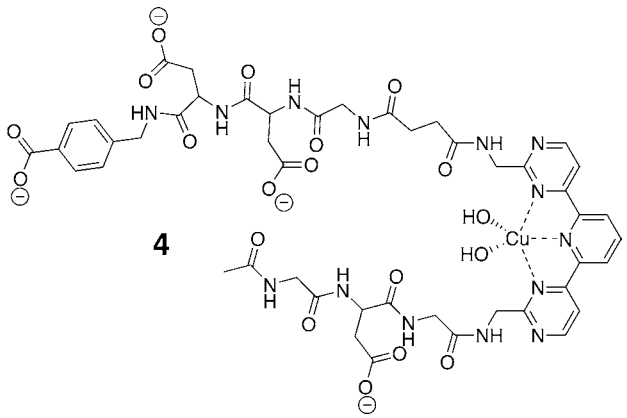
bidentate ligation with the metal center, but the proximally located guanidinium appendages imparted selectivity for aspartic acid. In a water/methanol solution **3** bound aspartic acid with an association constant of 1.5×10^5 M^{-1}.



3

We now describe the development of a tweezer-like receptor with a rigid backbone and two variable peptide arms preorganized for binding through a copper center. It is well documented that histidine in the N-terminal position of a peptide binds metals well through its terminal nitrogen and imidazole nitrogen, which is analogous to histamine coordination.[9] Moreover, cysteine, as well as methionine, can act as an excellent metal ligand. The current work shows the synthesis and selective recognition properties of the new receptor **4** for short peptides N-terminating in histidine, cysteine, and methionine, due to the favorable copper interaction. The backbone of receptor **4** is a polyaza tricyclic derivative of 2,2′:6,2″-terpyridines.[10] We also demonstrate that the selectivity of the tripeptide recognition can be modulated by peptide arms of the receptor. We specifically included acidic amino acid residues into the arms of the receptor to enhance binding via ion-pairing.[11]



4

The synthesis commenced with *tert*-butoxycarbonyl protection (Boc) of 2-aminoacetonitrile hydrochloride followed by condensation with ammonia to form the carboxamidine **7** in good yield (Scheme 1).[12] Further, the bisenaminone **10** was produced from Bredereck's reagent and 2,6-diacetylpyridine.[13] The backbone of the receptor, **11**, was formed through condensation of **7** and **10** in sodium ethoxide in marginal yield. Removal of the Boc protecting groups with trifluoroacetic acid afforded the diamine **12**. Incorporating the diamine into solid-phase synthesis required the mono-Fmoc protection of the diamine backbone. The single protection allows for coupling to the first peptide arm growing from the resin, followed by deprotection of the second amino group and subsequent coupling to the first amino acid residue of the second peptide arm. Thus, the diamine was singly protected to give the mono-boc **13** in good yield. Protection of the free amine with Fmoc, followed by subsequent Boc removal, gave the mono-Fmoc **15** backbone portion of the receptor.

Solid-phase synthesis was completed using 1-hydroxybenzotriazole (HOBt), 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), *N*-methylmorpholine (NMM), and 4-aminomethylbenzoyl 2-chlorotrityl resin.[14] Three amino acids, aspartic acid, aspartic acid, and glycine, were placed on the resin using the Fmoc protocol. After removal of the final Fmoc group with piperidine, the amine was coupled to the linker group, a monofluorenyl methanol (Fm)-protected succinic acid.[15]

The Fm group was removed leaving a free carboxyl group. Under coupling conditions, the mono-Fmoc diamine **15** was added to the growing peptide. The Fmoc group was removed, and subsequently three more amino acids were added; glycine, aspartic acid, and acetyl protected glycine. A representative coupling step is shown in Scheme 1.

The binding studies were carried out in a water/methanol solution (1:1; buffered with 100 mM HEPES at pH = 7.4). Upon addition of an amino acid (e.g., L-His) or tripeptide (e.g., L-His-L-Lys-L-Lys) to a constant ligand−metal concentration, there was an overall hypsochromic shift, and three isosbestic points emerged (λ = 330, 322, and 298 nm). Using the absorption data obtained at 314 nm, binding constants were calculated using a 1:1 binding algorithm.[16] Figure 1

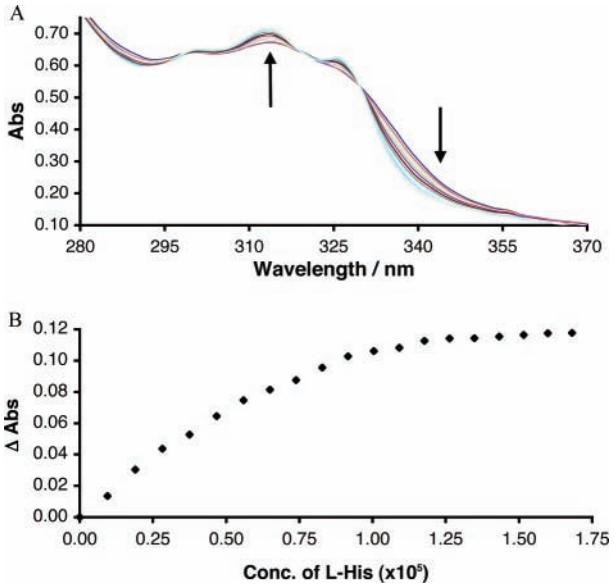


Figure 1. (A) Addition of L-His to a constant concentration of **4** (0.10 mM). (B) Binding curve (1:1) for the addition of L-His to **4**.

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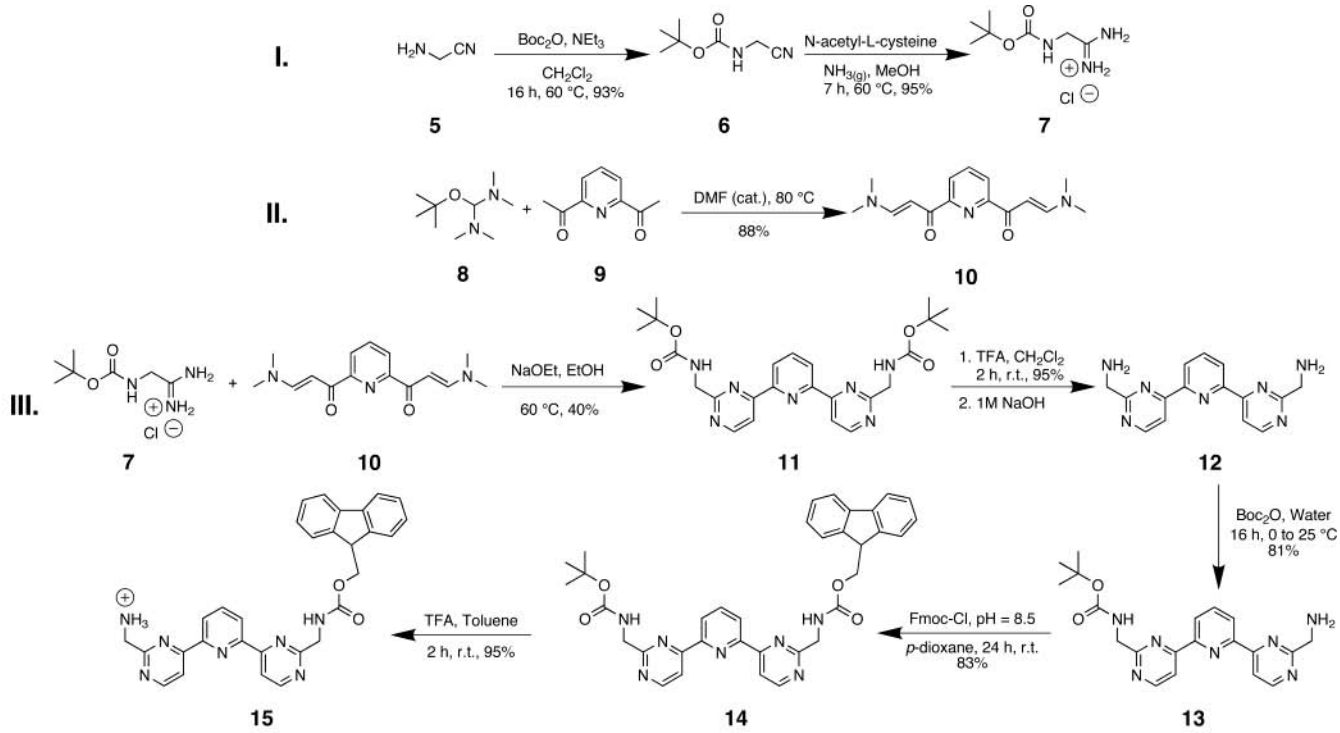
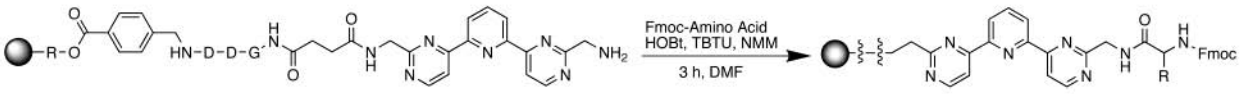
Scheme 1



Representative Solid-Phase Coupling:



R = trityl group of the resin
D = aspartic acid
G = glycine

shows a typical UV spectrum and 1:1 binding curve as given by the titration of L-His to the copper complex. Table 1 lists association constants for amino acids, protected amino acids, and tripeptides. From Table 1, it is evident that those amino acids that are good copper ligands bound well to the receptor complex. Histidine, cysteine, and methionine, with their coordinating imidazole nitrogen, thiol, and thioether, respectively, bound the copper complex.

The association constants of the protected peptides show that amino acids bound through their amino terminus (Table 1). When histidine was acylated at its amino terminus, no binding occurred. However, the methyl ester of histidine with

Table 1. Binding Constants (M^{-1}) of **4** with Different Amino Acids, Protected Amino Acids, and Tripeptides (Measured at 25 °C)

analyte	binding constant
Ac-L-His	0
L-His-OMe	1.0×10^5
L-His	1.2×10^5
D/L-Cys	8.0×10^4
L-Met	3.0×10^3
L-Val	0
Gly	0
L-His-L-Lys-L-Lys	1.0×10^6
L-His-Gly-Gly	1.5×10^4
Gly-Gly-Gly	0
L-Cys-L-Lys-L-Lys	3.0×10^5
L-Cys-Gly-Gly	5.0×10^2
L-Met-L-Lys-L-Lys	1.0×10^5
L-Met-Gly-Gly	2.5×10^4
L-Lys-L-Lys-L-His	0

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a free amino terminus bound ($1.0 \times 10^5\ M^{-1}$), as did histidine ($1.2 \times 10^5\ M^{-1}$). Nonchelating amino acids such as L-Val and Gly had no interaction with the receptor.

The association constants of the tripeptides fit our hypothesis that a metal ligand such as in histidine, cysteine, or methionine with two additional amino acids with molecular recognition sites that complement our receptor would bind well. For example, when a tripeptide terminated in the carboxyl group of histidine followed by two lysines, no binding occurred. Yet, N-terminal His, with two appended Lys, bound very well ($1.0 \times 10^6\ M^{-1}$). Similarly, L-Cys-L-Lys-L-Lys and L-Met-L-Lys-L-Lys bound well (3.0×10^5 and $1.0 \times 10^5\ M^{-1}$, respectively). There is an increase in affinity by a factor of near 10−30 due to the ion-pairing interactions between the host and guest peptide residues. In contrast, the His-, Cys-, and Met-Gly-Gly analogues all dropped in affinities by about 100 fold. Therefore, our data illustrate that chelation to the metal is paramount for the binding, but it can be enhanced by including complementary ion-pairing interactions.

In summary, we have shown that cooperativity in binding peptides can be achieved using a receptor possessing a metal-chelating site and ion-pairing sites. In specific, N-terminal metal-chelating amino acids appended to basic amino acids bound with enhanced affinities over metal-chelating and ion-pairing alone. We are now turning this work toward the creation of combinatorial libraries for differential recognition of peptides.

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Supporting Information Available: Experimental procedures and characterization for **11**−**15**, representative solid-phase coupling conditions, and the binding curve for HKK. This material is available free of charge via the Internet at http://pubs.acs.org.

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