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Unnatural multidentate metal ligating α-amino acids

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Abstract—A family of penta- and hexadentate metal ligating α-amino acids, suitably protected for Fmoc solid-phase chemistry, has been prepared. These residues incorporate the mono-amides of ethanolaminetriacetic acid, ethylenediaminetriacetic acid, and ethylenediaminetetraacetic acid as side chains. Side chains are tethered varying distances (*n*) from the Cα-carbon to allow metal binding events to occur at distinct distances from the peptide backbone. These residues are designed to allow the facile installation of metal chelates along a peptide backbone.

At functional metal sites within proteins, the ligands are provided, by either the amide backbone or more generally, the side chains of amino acid residues. Natural metal binding residues such as histidine, methionine, cysteine, aspartic, and glutamic acids individually act as low affinity mono- or bidentate ligands in the absence of a protein scaffold.[1,2] However, evolution of protein structure has culminated in exquisite protein folds that offer secondary structural elements and outer sphere interactions, such as hydrogen bonding, that uniquely position the side chains of ligating residues.[3–5] These nonlocal structural attributes, contributed from the entire protein scaffold, result in high affinity metal binding sites.

The de novo design of peptide and protein scaffolds that bind metal ions in predictable geometries using only naturally occurring amino acids is a field making notable progress, but is extremely difficult.[6–9] In order to design a high affinity site a priori, not only does one need to be concerned with the coordination sphere of the metal binding site, but equally important is the overall three dimensional structure of the entire protein that will serve to position the ligating side chain ligands.

The use of metal binding peptides and proteins is gaining prominence in materials science.[10–14] We are interested in fabricating novel biomaterials from self-assembling peptides[15–20] and are beginning to explore the possibility of using this mechanism to prepare metal-containing materials for use in microfluidics and sensor fabrication. Successful preparation of these materials relies on our ability to incorporate metal ions at precise sequential positions within each peptide comprising the assembly. Metal binding sites need to be incorporated within a given peptide reliably, affording metal complexes of significant stability. In addition, the ability to transpose a given metal binding site into any peptide would enable the preparation of focused libraries of metal-binding self-assembling peptides. Designing such sites using only naturally occurring amino acid residues would be a slow and daunting task.

Here, we report the synthesis of a family of amino acids that contain penta- and hexadentate aminocarboxy side chain ligand (Scheme 1) compounds **11**–**19**. Each amino acid represents an autonomous metal binding site capable of binding one metal ion without the aid of other residue side chains elsewhere in the sequence. Three classes of residues have been prepared. The first, residues **11**–**13**, and the second class, residues **14**–**16**, display pentadentate ligands as side chains, the mono-amides of ethanolaminetriacetic acid, and ethylenediaminetriacetic acid, respectively. The third class, comprising residues **17**–**19**, displays a hexadentate ligand, the mono-amide of ethylenediaminetetraacetic acid (Scheme 1—ligating atoms are shown in bold for residues **11**–**13** for reference). By varying the ligation number and identity of ligating atoms, each class should form metal complexes characterized by distinct stability constants and kinetic labilities. Within each class of residue, the number of

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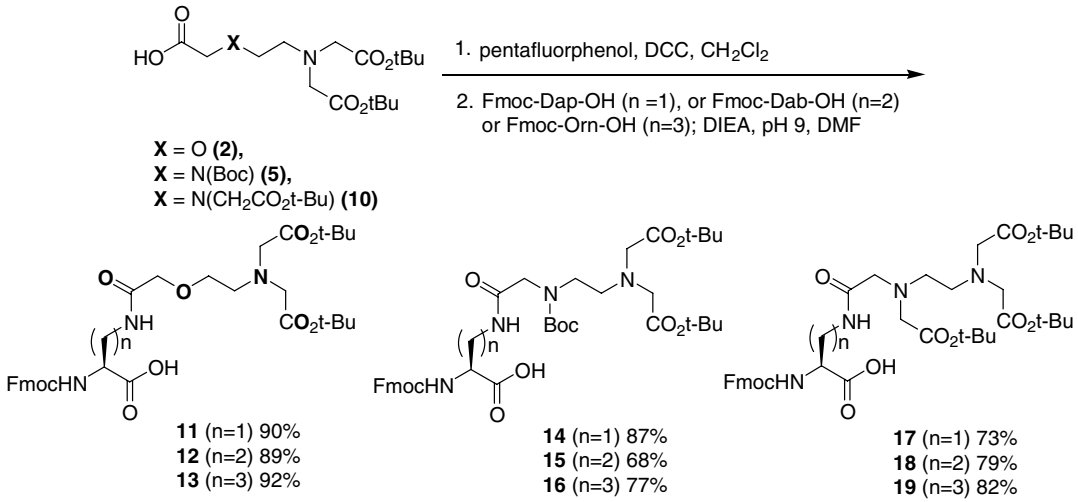


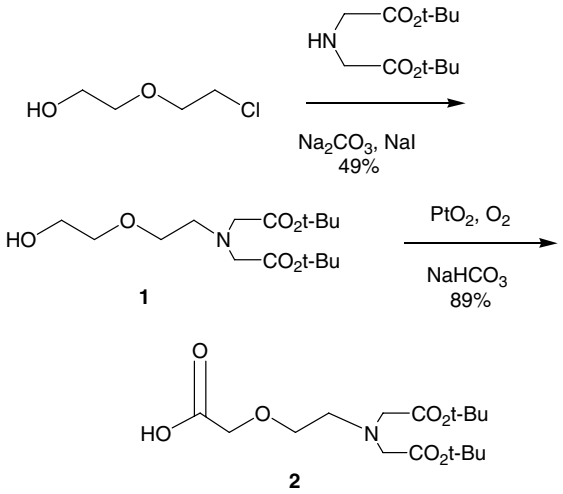
Scheme 1.

methylene groups separating the ligand from the Cα-carbon of the amino acid is varied ($n = 1$–3) so that the distance between the metal complex and the peptide backbone can be controlled. The residues have been Fmoc-protected so that standard solid phase peptide synthesis can be employed to easily prepare peptides containing one or multiple copies of any of these desired metal binding residues.

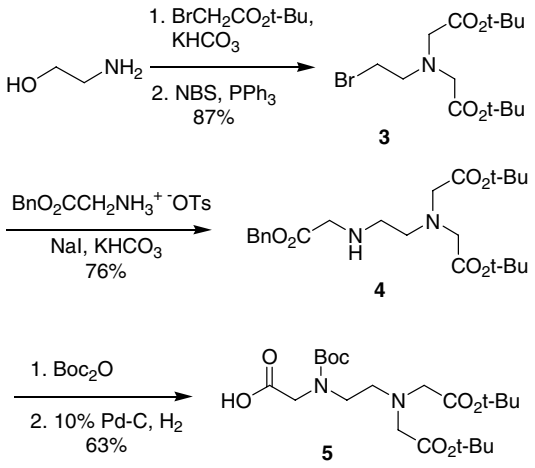
Historically, polycarboxylate ligands have found wide applications in industrial, medical, and agricultural settings, due to their ability to form metal chelates with high binding efficiency.[21] We chose three polycarboxylates as side chains that should bind a wide range of transition metals with differing affinities. For example, the well-characterized hexadentate ligand ethylenediaminetetraacetic acid[22,23] (precursor to the side chains of residues **17**–**19**), binds Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cr^{3+} with 10^{14}–10^{23} M^{-1} affinity, with Cr^{3+} forming the most thermodynamically stable chelate. In comparison, the pentadentate ligand, ethylenediaminetriacetic acid (precursor to the side chains of residues **14**–**16**) binds to Cr^{3+} with 10^{6} M^{-1} affinity.[24] Lastly, replacing one of the ligating nitrogens with an oxygen donor affords a side chain that should demonstrate preference for binding harder metal ions (residues **11**–**13**).

A facile convergent synthesis is reported that couples the pentafluorophenol active esters of mono-acids **2**, **5** or **10** to *N*-α-Fmoc-diaminopropionic acid (Fmoc-Dap), *N*-α-Fmoc-diaminobutyric acid (Fmoc-Dab) or *N*-α-Fmoc-ornithine (Fmoc-Orn) (Scheme 1). Active esters of **2**, **5**, and **10** are prepared via dicyclohexylcarbodiimide (DCC) activation of the corresponding carboxylic acids in the presence of pentafluorophenol and are used directly in the subsequent coupling reaction. Commercially available Fmoc-protected fragments react quickly (within 2 h) and cleanly with 1.2 equiv of active ester under basic solution conditions (pH 9) resulting in the formation of compounds **11**–**19** in good to excellent yield (see Supplementary data for detailed experimentals). For example, compounds **11**, **12**, and **13** were produced in 90%, 89% and 92% yield, respectively, from mono-acid **2**. Ligand **2** was prepared by first coupling 2-(2-chloroethoxy)ethanol to di-*t*-butyl iminodiacetate in the presence of sodium iodide affording di-*t*-butyl protected **1** in 49% yield (Scheme 2). Platinum oxide-assisted oxidation of **1** yields the corresponding mono-acid **2** in 89% yield.

The coupling of **5** to Fmoc-Dap, -Dab or -Orn (Scheme 1) affords residues **14**–**16** in 87%, 68%, and 77% yield, respectively. Scheme 3 provides the synthetic route for the *N*-Boc, di-*t*-butyl ester protected ligand **5**. Briefly, treatment of ethanolamine with *t*-butyl bromoacetate followed by bromination affords **3** in 87% yield for the two steps. Next, bromine is displaced by the tosylate salt of glycine benzyl ester yielding **4** in 76% yield. In concurrent reactions, the secondary amine of **4** is *N*-Boc protected using *t*-butoxy carbonyl anhydride and the benzyl ester is de-protected via hydrogenation to afford **5** in 63% yield.

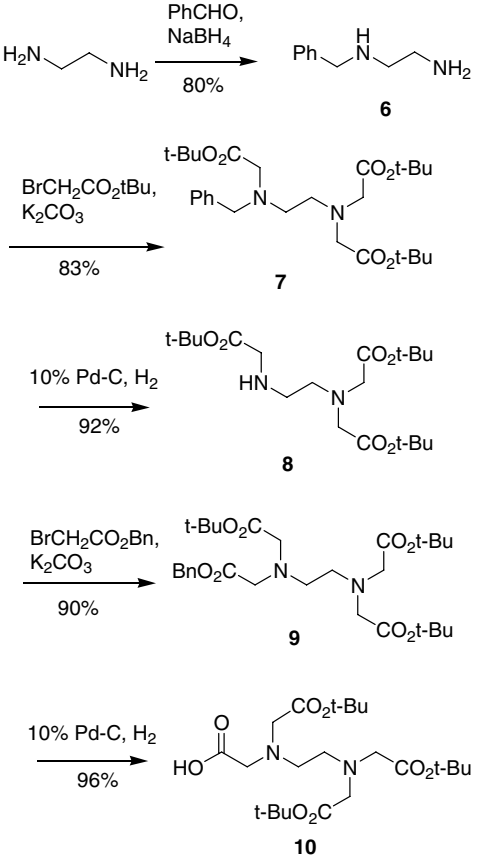


Scheme 2.



Scheme 3.

Finally, coupling the active ester of **10** to Fmoc-Dap, -Dab, or -Orn afforded **17**, **18**, and **19** in 73%, 79%, and 82% yield, respectively. Precursor **10** was prepared via an optimized procedure originally reported by Johannes et al.[25] (Scheme 4). Here, careful reductive amination of ethylenediamine affords mono-amine **6** in 80% yield. The benzyl protecting group of **6** allows an orthogonal protection strategy to be used to install the mono-free acid of **10** with the remaining three carboxylates suitably protected. Diamine **6** is then reacted with excess *t*-butyl bromoacetate to yield tri-functionalized **7** in 83% yield. Following this step, the orthogonal benzyl group was selectively removed by hydrogenation using 10% palladium on carbon. The resulting secondary amine **8** was reacted with benzyl bromoacetate to afford **9** in 90% yield, which was subsequently hydrogenated to finally yield mono-acid **10** in 96% yield.



Scheme 4.

In summary, a family of metal chelating amino acids has been prepared by reacting the active esters of suitably protected polycarboxylate ligands with commercially available Fmoc-protected Dap, Dab, and Orn residues. The synthesis of these unnatural residues, as well as their synthetic precursors, proceeds with good to excellent yields. We are currently utilizing these penta- and hexadentate residues in the fabrication of metal-containing, self-assembled peptide-based materials.

Acknowledgement

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Supplementary data

Experiments and characterization spectra (MS, ^{1}H NMR and ^{13}C NMR) for all prepared compounds are available in the Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.06.128.

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