Organization of Amino Acids Using a Metallotriazacyclononane Template

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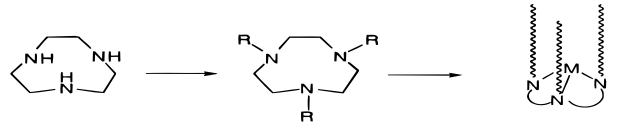
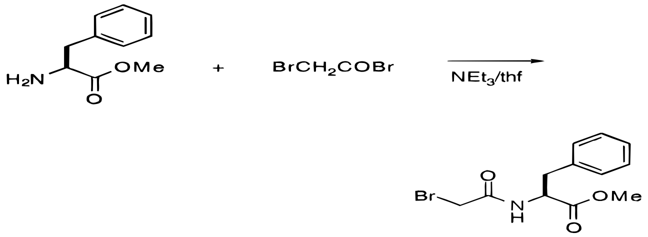
Like disulfide bonds, chelating metal ions that covalently bridge amino acids from discontinuous regions of a polypeptide can profoundly influence protein folding. Metal ions have also been shown to induce folding of short peptides into secondary structural elements such as α-helices,[1] β-turns,[2] and β-sheets,[3] which in turn may serve as folding templates in longer polypeptides. The metals themselves could also conceivably participate in the stabilization of folded peptides by capping their N- or C-termini or as templates for self-assembling[4] peptides. Our interest in "metal ion clips" that induce folding in peptides leads to this report of the effect of metal binding to an N-terminal peptide cap containing an unnatural metal-binding ligand.

The tridentate macrocycle 1,4,7-triazacyclononane (**1**) has become a staple ligand in coordination chemistry owing to its selective facial coordination, high affinity for transition and main group metals, and chemical stability of both itself and its complexes.[5] A number of N-functionalized derivatives[5] **2** have been made from **1**, including acetates (R = $-CH_2CO_2H$), acetate esters ($-CH_2CO_2Et$), sulfonates (e.g., $-CH_2CH_2SO_3H$), alcohols ($-CH_2CH_2OH$), phosphates ($-CH_2PO_3H_2$), and amines (e.g., $-CH_2CH_2NH_2$ and $-CH_2$pyr). However amino acid derivatives (R = $-CH_2CONHCH$\{side chain\}CO_2Me) do not yet appear to have been reported.

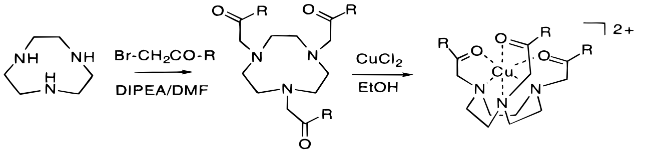


1 **2** **3**

Our objective was to functionalize the metal-binding ligand (**1**) with three amino acids (**2**, R = $-CH_2CONHCH(CH_2Ph)CO_2Me$), in preparation for construction of a three-helix peptide bundle (**3**, R = 10−15 amino acid sequence). Three helix bundles are reported in several protein structures.[6] We expected[5] **2** to bind to metals through its three tertiary amine nitrogens but were interested to discover whether the amino acids would also bind to the metal, which of the amide atoms (oxygen or nitrogen) would coordinate and if they would form five-membered rings with the metal, and whether all three amino acids would be constrained by the metal to orientate in the same direction. The metal "clip" might thus assist peptide association into three helix bundles (**2** → **3**).



The N-terminus of of the methyl ester of L-phenylalanine was acylated by reaction with bromoacetyl bromide in THF containing triethylamine. The resulting bromoacetylated amino acid was then used to alkylate all three nitrogens of 1,4,7-triazacyclononane (**1**) to form the N-functionalized macrocycle **2** (R = $-NHCH(CH_2Ph)CO_2Me$). This molecule reacts with $CuCl_2$ and $ZnCl_2$ in alcohol to form respectively yellow (**3a**) and white (**3b**) complexes which crystallized on addition of diethyl ether. Elemental compositions[7] were consistent with the formulations $[Cu(\mathbf{2})][CuCl_4]$ and $[Zn(\mathbf{2})][ZnCl_4]$, respectively.



1 **2** **3a**

To discover the nature of the coordination of **2** to the metal ion, we determined[7] an X-ray crystal structure of $[Cu(\mathbf{2})][CuCl_4]$. Figure 1 identifies the connectivity of the component non-hydrogen atoms for the $[Cu(\mathbf{2})]^{2+}$ cation. The CuN_3O_3 coordination sphere has distorted octahedral geometry. The triazacyclononane component of **2** is bonded facially to Cu through its three amine nitrogen atoms, while the other three donor atoms are *oxygen* atoms of uncharged amides derived from the bromoacetyl groups. Bond lengths and angles involving copper[8] are as expected,[5] with variations between the Cu−N and between Cu−O bonds of up to 0.1 Å. The three O−Cu−O angles are 90 ± 2°, while the three N−Cu−N angles are 82.5

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(7) Anal. Calcd for **3a**, $C_{42}H_{54}Cl_4N_6O_9Cu_2$: C, 47.8; H, 5.2; N, 8.0. Found: C, 47.2; H, 5.3; N, 7.8. Calcd for **3b**, $C_{42}H_{54}Cl_4N_6O_9Zn_2$: C, 47.6; H, 5.1; N, 7.9. Found: C, 47.0; H, 5.0; N, 7.5. X-ray structural data for **3a**, $[Cu(\mathbf{2})][CuCl_4]$: $C_{42}H_{54}Cl_4Cu_2N_6O_9$, fw = 1055.83, T = 20.0 °C, orthorhombic, $P2_12_12_1$ (No. 19), a = 13.552(4) Å, b = 16.098(4) Å, c = 22.772(7) Å, V = 4967(2) Å^3, Z = 4; R (R_w) = 0.040 (0.035), D_{calc} = 1.412 g/cm^3, F_{000} = 2184.00, μ(Mo Kα) = 11.27 cm^{-1}.

(8) Selected interatomic bond lengths (Å): Cu(1)−O(1), 2.064(4); Cu(1)−O(2), 2.177(4); Cu(1)−O(3), 2.064(5); Cu(1)−N(1), 2.184(5); Cu(1)−N(2), 2.135(6); Cu(1)−N(3), 2.079(6); O(1)−C(8), 1.244(7); C(8)−N(4), 1.324(8); O(2)−C(20), 1.235(7); C(20)−N(5), 1.314(7); O(3)−C(32), 1.242(8); C(32)−N(6), 1.311(8). Selected bond angles (deg): O(1)−Cu(1)−O(2), 89.4(2); O(1)−Cu(1)−O(3), 91.3(2); O(2)−Cu(1)−O(3), 91.5(2); N(1)−Cu(1)−N(2), 81.6(2); N(1)−Cu(1)−N(3), 82.4(2); N(2)−Cu(1)−N(3), 83.4(2); O(1)−C(8)−N(4), 122.9(6); O(2)−C(20)−N(5), 123.2(7); O(3)−C(32)−N(6), 123.0(7).

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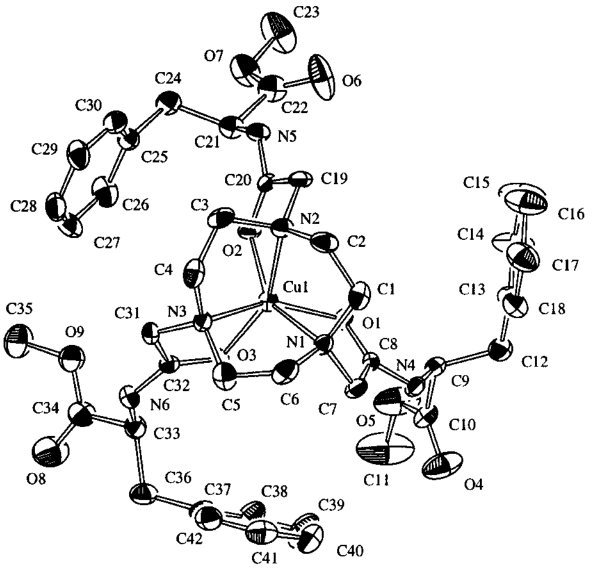

Figure 1. Thermal ellipsoid diagram of the cation of **3a** with labeling of selected atoms. Ellipsoids show 25% probability levels except for hydrogen atoms, which have been omitted for clarity.

± 1°. The carboxamides have normal C−N (1.31−1.33 Å) and C−O (~1.24 Å) bond lengths and O−C−N angles (~123 ± 0.2°) consistent with trans amide bonds.

The benzylic side chains of the phenylalanine are packed in a propeller-like arrangement that is anticlockwise (Figure 1), as reported for acetates (R = −OMe).[5] The amide main chains all project in the same direction, with interchain distances between the α-carbons of phenylalanines being 7 ± 0.1 Å. This compares favorably with the interaxial distances between α-helices in proteins (9 ± 3 Å)[9] and suggests the possibility of metal-induced three-helix bundle formation for longer peptides attached to the triazacyclononane scaffold.

The results here demonstrate that coordination of a metallotriazacyclononane template to three amino acids can be used to assemble their main chains in close proximity and orientate them in the same direction. The metal ion plays a crucial organizing role in pulling together the three amide groups attached to the triazacyclononane template. Such organization illustrates how N-capping of peptides with a metallotriazacyclononane could be used as a device to promote peptide association. For example, substitution of the amino acids with longer peptides may permit association into tertiary structures such as three-helix bundles.[10]

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Supporting Information Available: Text giving full experimental details for the synthesis of **2**, **3a**, and **3b** and the X-ray structure determination of **3a** and additional structural diagrams and listings of crystal data, X-ray experimental details, atomic coordinates, thermal parameters, distances, angles, and least-squares planes for **3a** (46 pages). Ordering information is given on any current masthead page.

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