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Synthesis of A Metal-Ligating Amino Acid Suitable for Solid Phase Assembly of Peptides

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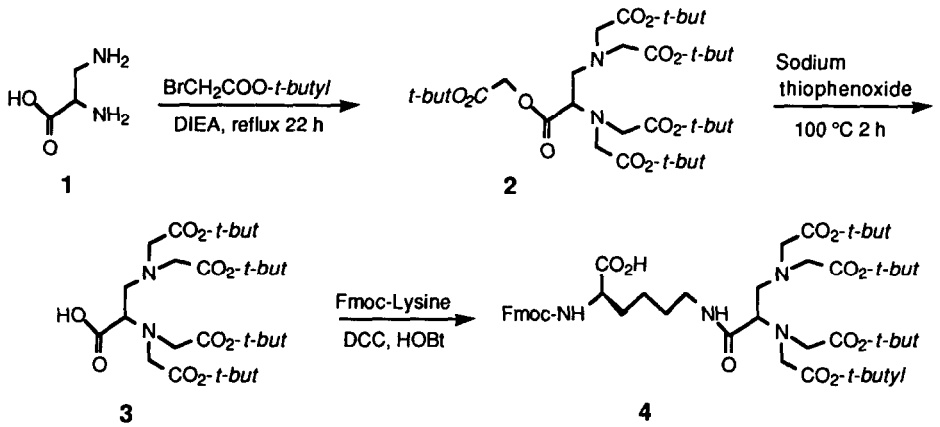
Abstract: The synthesis of an amino acid analog, N-α-Fmoc-N-ε-Tetrabutyl EDTA-L-Lysine, suitable for incorporating a strong metal binding site at any internal sequence position in a peptide is described.

Cleavage of DNA or RNA by metal chelates is an important new approach to characterize specific structural features of nucleic acids and their complexes in solution.[1] Recently, there has been considerable interest in the cleavage of proteins by metal ions or chelates bound at particular sites.[2,3] Site-specific cleavage of proteins is achieved by introducing a metal-binding site at one position in a polypeptide chain.[2] A reaction has been discovered by which proteins can be hydrolyzed by an analog of iron-EDTA in the presence of ascorbate and hydrogen peroxide.[4] Inducing proteolysis with metal chelates would permit mapping a site of interest by determining which individual peptide bonds are close to the metal site. Another important use of metal chelators is to prepare radiolabeled monoclonal antibodies for the early detection and therapy of cancer.[5] Syntheses of peptides carrying EDTA at N-terminus or proximal to C-terminus have been previously reported.[6,7] An EDTA analog of N-α-Boc-lysine amino acid has been recently synthesized.[8] With one exception,[7] all synthetic routes utilized one of the carboxylic arms of EDTA to anchor the chelating site to the peptides. This methodology has two limitations: (i) the metal binding moiety is not exactly EDTA and affinity cleavage of proteins using this chelate (ethylenediaminetriacetic acid) resulted in non-hydrolytic fragments,[3] (ii) stability of metal complexes could be a problem during the use of the chelate to prepare radiopharmaceuticals. Here we report a simple and straightforward synthesis of a modified lysine (**4**) for incorporation of metal-ligating functionality (ethylenediaminetetraacetic acid) at any desired position in a peptide.

N-α-*tert*-butyloxycarbonyl- (Boc) protected amino acids are used for Merrifield solid phase peptide synthesis.[9] There are two major concerns about this synthetic strategy: (a) repetitive TFA acidolysis in Boc-group deprotection could lead to acid catalyzed side-reactions, (b) cleavage and deprotection of peptides requires HF and specific laboratory set up which is not available to many researchers. Due to these concerns Fmoc (9-fluorenylmethyl carbamate) solid phase peptide synthesis was developed which employs N-α-Fmoc amino acids.[10] In this strategy, the Fmoc group is deprotected with piperidine and TFA is required only for the final cleavage and deprotection step. Compound **4** was designed to be compatible with the Fmoc solid phase peptide synthesis strategy.

Scheme I



The *tert*-butyl ester analogue of EDTA was synthesized in two steps as shown in Scheme I. The starting material **1** (5.0 g) was dissolved in 100 mL of acetonitrile and mixed with 5 equivalents of *tert*-Butyl bromoacetate and diisopropylethylamine (DIEA). The reaction mixture was refluxed for 22 hours, cooled, and the solvent was removed under reduced pressure. The residue was triturated with 350 mL of anhydrous ether stirred overnight and filtered. The filtrate was dried, dissolved in 200 mL toluene and washed five times with 0.1M phosphate buffer, pH 2.0. The organic phase was dried over molecular sieves and the solvent evaporated under reduced pressure (crude residue, 30.8 g). The intermediate compound **2** (30.08 g) was dissolved in 200 mL of DMF and 4.65 g of sodium thiophenoxide was added.[7, 11] The reaction mixture was heated at 100 °C for 2 hours, cooled to room temperature, and diluted with 300 mL toluene. After washing with 0.1M phosphate buffer, pH 2.0, the solvent was removed on a rotary evaporator (yellow colored oily residue, 28.18 g). The residue (1.0 g) was loaded on a silica gel column and eluted with 50 mL fractions of increasing polarity (*n*-

hexane and ethyl acetate). The desired product **3** was eluted with 1% (v/v) glacial acetic acid in ethyl acetate (yellow oily residue, 0.28 g). *^1H NMR* (500 MHz, $CDCl_3$) δ 3.7 (t, J = 7.4, 1H), 3.5 (m, 8H), 3.1 (d, J = 7.4, 2H), 1.5 (s, 36H). *IR* 1730 cm^{-1} (C=O, s). *MS* (FAB) calculated M+H for $C_{27}H_{49}N_2O_{10}$ 561, observed M+H 561. *TLC* (SiO_2, chloroform/methanol/acetic acid, 9:1:0.004) R_f 0.52.

The final compound **4** was prepared by using 1-hydroxybenzotriazole (HOBt) to make an active ester of EDTA analogue (**3**).[12] The tetrabutyl ester protected bifunctional chelating agent **3** (0.5 mmol) was dissolved in 3 mL of dichloromethane and 0.5 mmol of 1-hydroxybenzotriazole (dissolved in 1.5 mL of DMF) was added. The resulting solution was mixed with 0.5 mmol dicyclohexylcarbodiimide (DCC) dissolved in 1 mL methylene chloride. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2 h, followed by filtration. The filtrate was evaporated to dryness, and the activated ester was dissolved in 5 mL DMF and 2 mL methylene chloride. N-α-Fmoc lysine (0.6 mmol) was partially dissolved in 50 mL ethylene glycol monoethyl ether and added to the activated ester solution, followed by addition of 100 μL of diisopropylethylamine to bring the apparent pH to 8.0. The reaction was carried out overnight at room temperature with constant stirring. The reaction was stopped by removing the solvent under reduced pressure. The residue was taken up in ethyl acetate and excess N-α-Fmoc lysine was extracted with 10% citric acid. The organic phase was dried over molecular sieves and the solvent removed under reduced pressure to provide 90% of **4** as a dark yellow oil. This crude product was chromatographed on C_{18} reverse phase column, and eluted with 75% acetonitrile (in 0.1% aqueous trifluoroacetic acid) to give 82% of **4** as a yellow oily residue. *^1H NMR* (500 MHz, $CDCl_3$) δ 7.7 (d, J=7.3, 2H, aromatic), 7.6 (d, J=7.2, 2H, aromatic), 7.3 (m, 4H, aromatic), 4.1 (dd, J=4.9, 8.9, 1H), 3.8 (t, J=7.4, 1H) 3.6 (m, 8H), 3.2 (d, J=7.4, 2H), 2.7 (m, 2H), 1.4-1.9 (m, 6H), 1.6 (s, 36H). *IR* 1730 cm^{-1} (C=O, s). *MS* (FAB) calculated M+H for $C_{48}H_{71}N_4O_{13}$ 911, observed M+H 911. *TLC* (SiO_2, acetone/water, 7:3) R_f 0.77.

Summary. We have described a convenient and straightforward method to synthesize a metal-ligating amino acid suitable for introducing a strong metal binding site at any chosen position in a peptide. Compound **4** is designed to be compatible with N-α-Fmoc peptide synthesis strategy, and can easily be prepared on large scale. Through this methodology, flexible linkers of different lengths and containing various structures can be placed between the α-carbon backbone of peptides and metal binding moieties. These peptides will provide a variety of affinity cleaving reagents which can be directed against protein or nucleic acid targets. Therefore, these molecules can serve as an important tool to study protein folding, protein-protein and protein-nucleic acid interactions.

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