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SYNTHESIS OF A SYMMETRICALLY BRANCHED TEMPLATE FOR PARALLEL α-HELIX DIMERS

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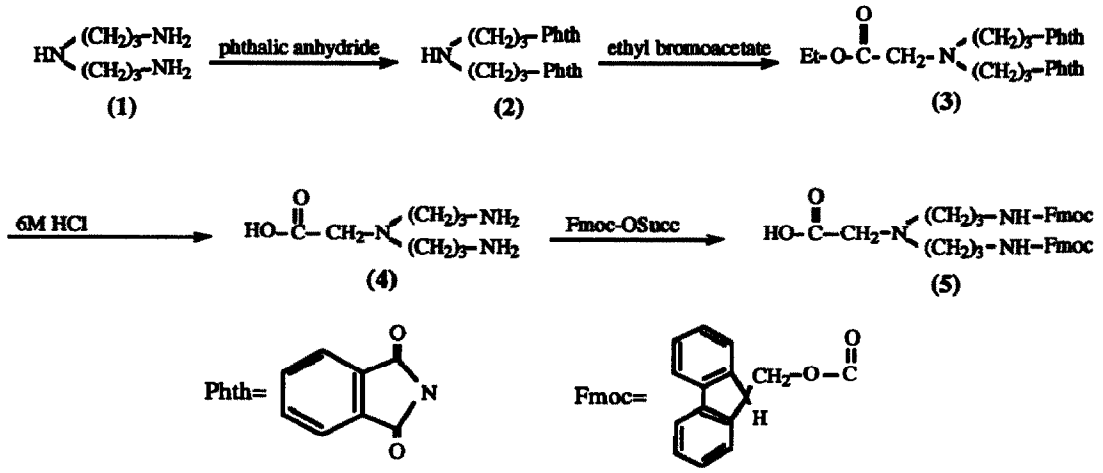
Abstract The synthesis of a symmetrical diamino acid is described for the assembly of parallel α-helices in solid phase peptide synthesis. This handle is designed to replace the C-terminal half of a class of DNA binding proteins, the Leucine Zipper.

Proteins have evolved into complex three dimensional structures that have specific biological functions. It is of great interest to be able to create or synthesise new molecules that mimic properties of the biologically active counterparts[1,2]. A major hurdle in the design of new peptides is the limited understanding of the correlation between protein primary sequence and tertiary structure[3]. In the study of the nature of protein folding many methods have been employed in the stabilization of helical and β-sheet structures in model peptides[2,4-7].

The Leucine Zipper class of proteins[8] consist of two parallel α-helices that associate to form non-covalent dimers. Each helix has a DNA binding domain at the N-terminus and a C-terminal "zipper" that holds the two helices together. A logical synthetic model to simplify and mimic the natural DNA binding protein is to covalently link the two identical binding domains together such that the dimeric structure is preserved without the leucine zipper. One method reported used a Cysteine cross linkage[9]. An alternative approach is to use the 2 amino groups of Lysine[10,11] as a branching point, however the unsymmetrical nature of Lysine is not ideal. Our approach decribed here is to replace the C-terminal "zipper" with a symmetrically branched handle (5).



Scheme

In this paper we describe the synthesis of a symmetrical diamino acid (**5**) for the simultaneous assembly of peptides (**Scheme**). Initial steps involve protection of the primary amino groups of (**1**) with phthalic anhydride[12] followed by alkylation of the secondary amine with ethyl bromoacetate. The ester and phthalimido groups were simultaneously removed by hydrolysis in 6M HCl. The final compound (**5**) was obtained after reaction of (**4**) with Fmoc-OSucc.

The application of compound (**5**) as a symmetrical handle in solid phase peptide synthesis (SPPS) was successfully illustrated with the synthesis of a small model peptide (**Figure 1**). Synthesis was performed on Rink[13] amide resin (0.62 mmol/g) using N-α-Fmoc protection for amino acids. Couplings were carried out in DMF using 2 equiv. of amino acid, 2.1 equiv. of BOP[14], 2.1 equiv. of HOBt[14] and 3.1 equiv. of DIPEA[14] per amino function. All reactions were complete within 30 min. Fmoc deprotection was carried out in 50% piperidine/DMF (1 x 1 min, 1x 5 min). The N-terminus of the final peptide was left protected with the Fmoc group. The peptide resin was cleaved with Trifluoroacetic acid/ethanedithiol/thioanisole/phenol/water (16:1:1:1:1)[15] for 1.5 hours at room temperature to give the crude peptide. The peptide was purified by reverse phase HPLC[16] and the identity confirmed by the MW as measured by electrospray mass spectrometry (ESMS)[17] (**Figure 2**).

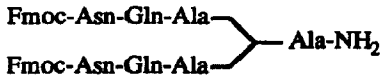


Figure 1. Sequence of the model peptide.

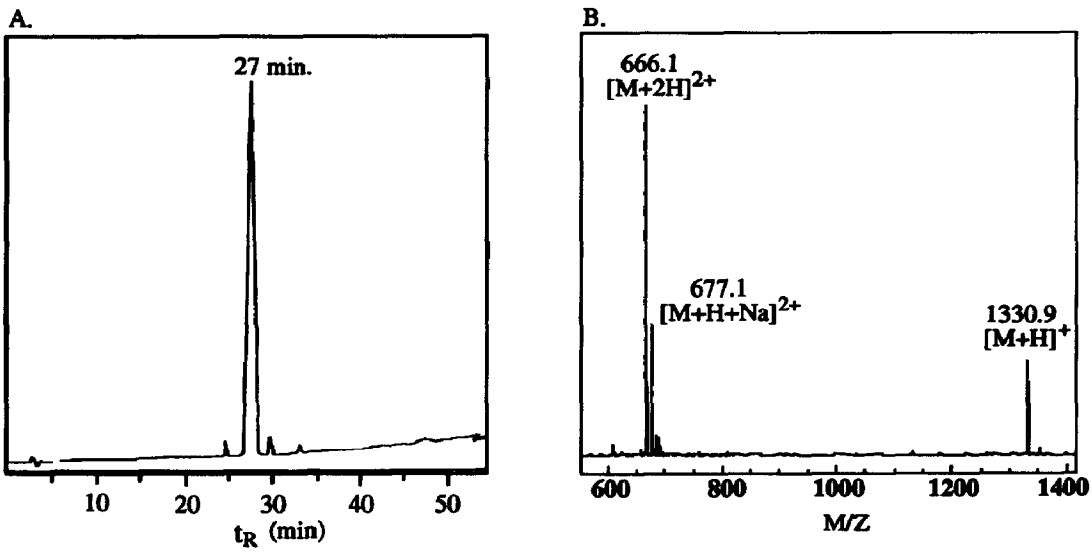


Figure 2. A. HPLC of the model peptide (t_R 27 min) after cleavage and B. ESMS of the purified peptide.

Compound (5) may be used in an identical way to the standard amino acids in SPPS to synthesise a range of symmetrical polypeptides. The successful synthesis of the model peptide illustrates compound (5) can be used for the synthesis of parallel α-helices.

Experimental

N,N-bis-(3-aminopropyl)amine (1) (10g, .08 mol) was dissolved in chloroform (200 mls) and Phthalic anhydride (2.1 equiv., 25 grams) in chloroform (100 mls) was added dropwise to the warm amine solution. On completion of the addition of phthalic anhydride the mixture was refluxed for 36 hours. The chloroform was removed *in vacuo* and the oily residue was recrystallised from ethanol / aq. HCl (.01M) to give a white solid. Yield 51%. mp. 207-209 °C. ESMS: found: $[M+H]^+$ 392.0, calc. 391.43.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27, m, (4H); 3.01, b s, (4H); 3.84, t, J=6.5Hz, (4H); 7.73, 7.82, m, (8H); 9.69, b s, (2H). ^{13}C NMR ($CDCl_3$, 75.45 MHz) δ 24.95; 34.43; 45.10; 122.69; 131.17; 133.61; 167.52.

N,N-bis-(3-phthaloylaminopropyl)amine (2) (10g, .026 mol), ethyl bromoacetate (1.5 equiv.) and DIPEA (2.5 equiv.) were heated in DMF (30 mls, 70°C) for 48 hours. The DMF was removed *in vacuo* and the remaining oil was chromatographed through a silica gel column using 30% THF / hexane. The product was recrystallised from DCM / ether / hexane. Yield 82%. mp. 93-97 °C. ESMS: found $[M+H]^+$ 477.9, calc. 477.52.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24, t, J=7.1Hz, (3H); 1.82, quin, J=7.1Hz, (4H); 2.72, t, J=6.9Hz, (4H);3.76, m, (4H); 4.12, quart, J=7.2Hz, (2H); 7.71, 7.82, m , (8H). ^{13}C NMR ($CDCl_3$, 75.45 MHz) δ 13.82; 26.14; 35.63; 50.95; 53.49; 59.71; 122.63; 131.69; 133.33; 167.83; 170.75.

N,N-bis-(3-phthaloylaminopropyl)glycine ethyl ester (3) was hydrolysed in 6M HCl for 12 hours. The solution was concentrated *in vacuo* to small volume and the phthalic acid was filtered off. The product was triturated with ethanol and the supernatant was decanted to give a white oil. This was used without further purification.
^{1}H NMR (D_2O 300 MHz) δ 1.99, m, (4H); 2.93, m, (4H); 3.22, m, (4H), 3.79, s, (2H).

N,N-bis-(3-aminopropyl)glycine (4) and $NaHCO_3$ (4 equiv.) were dissolved in water. This solution was added dropwise to a solution of Fmoc-OSucc. (2.1 equiv.) in dioxane / water. Reaction was monitored by the disappearance of the amino acid (spot test with ninhydrin). The dioxane was removed and the *N,N* bis-(3-fluorenylmethoxycarbonyl-aminopropyl)glycine (5) was extracted with chloroform. Purification was performed by silica gel chromatography using 5% methanol / chloroform to give an amorphous solid after drying *in vacuo*. ESMS: found $[M+H]^+$ 634.2, calc. 633.74.
^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.53, m, (4H); 2.54, m, (4H); 4.23, m, (6H); 7.3, t, J=7.4Hz, (4H); 7.41, t, J=7.4Hz, (4H); 7.43, m (2H); 7.66, d, J=7.4Hz, (4H); 7.86, d, 7.4Hz, (4H). ^{13}C NMR (DMSO-d_6, 75.45 MHz) δ 23.86; 37.51; 46.66; 51.77; 52.69; 65.29; 120.09; 125.01; 127.02; 127.56; 140.68; 143.80; 156.15; 167.56.

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12. Phthalic anhydride was recrystallised from chloroform. The insoluble phthalic acid was filtered off while hot.
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14. Abreviations. BOP: Benzotriazole-1-yl-oxy-tris-Pyrrolidino-Phosphonium-Hexafluorophosphate, HOBt: N-Hydroxybenzotriazole, DIPEA- Diisopropylethylamine.
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16 HPLC conditions. Solvent A. 0.1% TFA in water. Solvent B. 0.07% TFA/90% AcCN/water. Delta Pak C-18, 15μ, 100Å, (7.8mm x 300mm), Waters Associates. Linear gradient 30% B - 100% B in 35min. 3ml/min.
17. ESMS was performed on a VG BIO Q mass spectrometer.

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