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A New Strategy Applied to the Synthesis of an α-Helical Bicyclic Peptide Constrained by Two Overlapping i, i+7 Side-Chain Bridges of Novel Design

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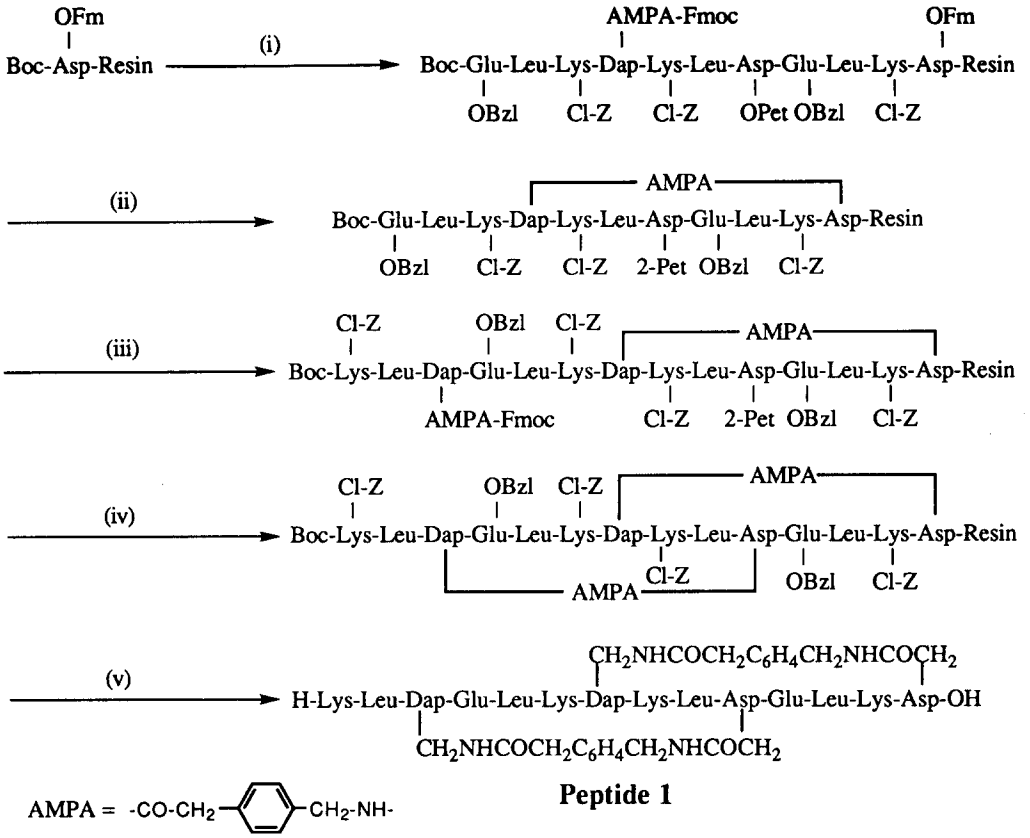
Abstract: A conformationally constrained, bicyclic, 14-residue peptide containing two overlapping i, i+7 side-chain bridges has been synthesized. The design of the side-chain linkage, which is built around a *p*-substituted benzene ring for rigidity, and the solid-phase approach applied to the peptide synthesis, are both new. A Boc/Benzyl peptide chain assembly method was combined with Fmoc/OFm orthogonal side-chain deprotection and solid-phase cyclization to form the first side-chain lactam bridge. A 2-(2-pyridyl)ethyl group (2-Pet), activated by methylation with CH_3I in DMF, was then used in combination with Fmoc to allow a second orthogonal side-chain deprotection and solid-phase cyclization to form the second bridge. The circular dichroism spectrum of the product indicates that it is highly helical.

Conformationally constrained peptidomimetics are valuable tools for identifying receptor-bound peptide conformations and in the rational design of biologically active small molecules. Several approaches have been reported for constraining and stabilizing α-helical peptides, including incorporation of amino acids with a high helix-forming propensity[1], the use of helix-stabilizing caps[2], incorporation of salt bridges,[3] metal chelates,[4] or amide bonds[5] that bridge i and i+4 residue positions in the peptide chain, and a disulfide bond that bridges i and i+7 positions.[6] These attempts have so far met with limited success, primarily due to the unfavorable nature of helix initiation[1,7] and the flexible nature of these bridges. Recently, we have developed a novel and rigid side-chain bridge in which (S)-1,3-diaminopropionic acid (Dap) and Asp side chains in i and i+7 positions in the peptide chain, respectively, are linked by condensation with 4-(aminomethyl)phenylacetic acid (AMPA).[8] The circular dichroism (CD) spectra of model peptides incorporating this bridge show high helicity in water at 0-80°C. We have also been successful in stabilizing α helix in a simple hexapeptide model structure, by using a bicyclic design consisting of overlapping i, i+4 side-chain bridges.[9] We have now investigated the synthesis of a model peptide designed to combine both of these successful strategies for α helix stabilization. The synthetic target is a 14-residue, bicyclic peptide (peptide **1**, Scheme 1) incorporating two of the new i, i+7 side-chain bridges (Dap^i-AMPA-Asp^{i+7}) placed in overlapping positions in the linear sequence. To obtain this compound, we used a new solid-phase approach of general and facile applicability.

The solid-phase synthesis of monocyclic, side-chain lactam-bridged peptides, using a Boc/benzyl strategy in combination with temporary Fmoc/OFm protection for the amine and carboxyl functionalities of the bridge is now well established.[5] However, the complete assembly of our target bicyclic, lactam-bridged peptide (**1**) on a solid support requires an extra level of orthogonal protection for the amine or carboxyl functionality on the C-terminal side of the second bridge. In search of a suitable protecting group for this functionality for the synthesis of peptide **1**, where the C-terminal linkage of the second bridge is the β-carboxyl group of Asp[10], we considered only high-yield reactions that would be generally compatible with peptide chemistry, and adaptable to use for amines or carboxyl side-chain protection, so that lactam-based bridges could be incorporated in either direction with equal facility. Kunz and Kessler and their co-workers[10] had synthesized amino acid 2-(2-pyridyl)ethyl esters (2-Pet esters) and found that the 2-Pet protecting group is stable to hydrogenolysis and treatment with trifluoroacetic acid and amines. However, after methylation of the pyridyl nitrogen with CH_3I in CH_3CN, the 2-Pet group was cleaved by a β-elimination mechanism under mild conditions with amines. Furthermore, a similar strategy for amine protection using the corresponding urethane derivative was also demonstrated. Unfortunately, the deprotection yields reported for these groups (69-92%)[10] were not reproducibly high enough for satisfactory application in solid-phase syntheses. We believed that the problem lay with the methylation step, reportedly performed in CH_3CN, which is slow because of charge buildup in the transition state and the products, and that this step could be further optimized by using alternative solvents for the reaction. In model studies using Boc-Asp(2-Pet)-OH, the effects of



Scheme 1. Solid-phase synthesis of bicyclic peptide **1**. Reagents and conditions: (i) coupling cycles for residues 13 through 4, (a) N^{α}-Boc deprotection: TFA/DCM (1/1), 30 min., RT, (b) coupling: 3 equiv. each Boc-Xxx-OH/ HBTU/HOBt + 6 equiv. DIEA in DMF/DCM (2/1), RT, until complete; (ii) (a) Fmoc/OFm deprotection: piperidine/DMF (1/4), 30 min., RT, (b) cyclization: HBTU/HOBt, 3 equiv. each, + 10 equiv. DIEA, in DMF/DCM (2/1), 12 h, RT; (iii) coupling cycles for residues 3 through 1, methods same as (i); (iv) (a) 2-Pet methylation: 10% (v/v) CH_3I in DMF, 48 h, RT, dark, (b) Fmoc/2-Pet deprotection: morpholine/DMF (3/7), (c) cyclization: HBTU/HOBt, 3 equiv. each + 10 equiv. DIEA, in DMF/DCM (2/1), 12 h, RT; (v) deprotection and cleavage: liquid HF/anisole/DMS (10/1/1, v/v/v), 0°C, 1 h.

different perdeuterated solvents on methylation by CH_3I (5% v/v) were investigated by ^{1}H-NMR. The resonance of the β-CH_2 of 2-Pet was significantly shifted upon methylation of the pyridyl nitrogen, and the extent of the reactions was monitored by measuring the areas of these peaks as a function of time (Figure 1). From this data, 2-Pet methylation rates were ordered: DMSO > DMF > CH_3CN. In methanol, no product was detected. Since DMSO effects the rapid oxidation of CH_3I, we chose to use DMF as the solvent for 2-Pet methylation in the solid-phase assembly of peptide **1** (Scheme 1).

Bicyclic peptide **1** was synthesized starting from a hydroxymethyl polystyrene/divinyl benzene resin, as described by Scheme 1. Boc-Asp(OFm)-OH was attached to the resin using N,N'-dicyclohexylcarbodiimide (DCC) and 4,4-dimethylaminopyridine (DMAP) in DCM. The peptide chain was assembled on the resin in the N-terminal direction, through residue 4, using the Boc/benzyl strategy, and incorporating the preformed bridging dipeptide unit Boc-Dap(Fmoc-AMPA)-OH (Scheme 2) into position 7. The first side-chain bridge

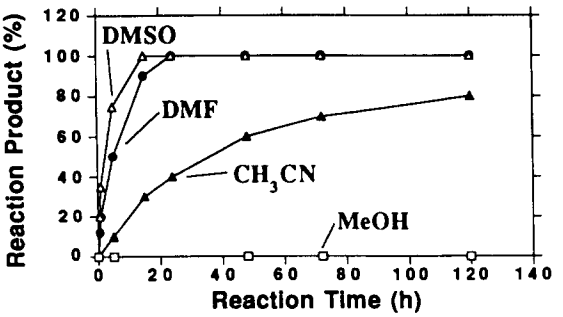
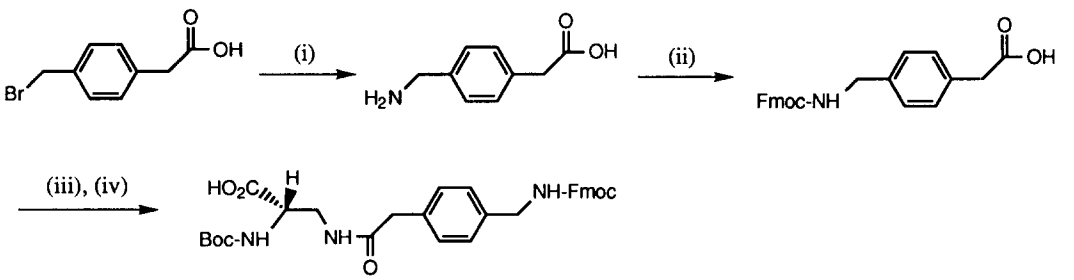


Figure 1. Solvent effects on methylation of Boc-Asp(2-Pet)-OH: Boc-Asp(2-Pet)-OH (6.69 mg, 0.02 mmol) was dissolved in 1.0 ml of pure deuterated solvent (0.02M) and 50 µl of CH_3I (5% v/v) was added into the solution. The solutions were shaken in the dark at RT. Percent product was calculated from the areas of the new ^{1}H-NMR resonance peaks for the β-CH_2 of the N-methylated 2-Pet group.



Scheme 2. Synthesis of Boc-Dap(Fmoc-AMPA)-OH: (i) NH_3 (2 M) in EtOH/acetone (4/1), 2 h, RT; (ii) Fmoc-OSu + DIEA in H_2O/CH_3CN (1/1), 2 h, RT (yield = 49% for steps i + ii); (iii) DCC + HOSu in dioxame/EtOAc (1/1), 20 h, RT; (iv) Boc-(L)-Dap-OH, 2.5% (w/v) $NaHCO_3$ in H_2O/dioxane (1/1), 10 h, RT (yield = 69% for steps iii + iv).

was then formed, in the presence of the Asp(2-Pet) group in position 10, by deprotection and coupling of the side-chain aminomethyl group of Dap(AMPA)[7] and the side-chain carboxylic acid group of Asp[14], essentially as described by Felix et al.[5] Assembly of the peptide chain through residue 1 was then completed, and the 2-Pet protecting group for Asp[10] was methylated using 10% (v/v) CH_3I in DMF for 48 h. The N-methylated 2-Pet group, and the Fmoc protecting group on Dap(AMPA)[3], were both removed using morpholine/DMF (3/7), and then the second cyclization reaction was carried out in DCM/DMF (2/3, v/v), as before. Subsequently, a Kaiser test was negative, indicating that the deprotection of 2-Pet had been complete. After the final deprotection and cleavage, peptide **1** was readily identified as the major peak on RP-HPLC (Figure 2), and was purified by a single RP-HPLC elution. Ion-spray MS: $[M+H]^+$ = 1903± 0.6 (calc. = 1903.3). Amino-acid analysis: Asx (2) 1.96, Glx (2) 1.92, Leu (4) 4.00, Lys (4) 3.82, Dap (2), 1.92, AMPA (2), 1.85.

The far-UV CD spectrum of peptide **1** was measured at pH 7.0 and 25 °C (Figure 3). The shape of this spectrum is similar to that expected for a high degree of helical structure. However, the intensities of the minima are lower than expected for α-helical peptides.[11] No significant CD signal was observed in the near UV (250 - 300 nm), indicating that the aromatic bridge structures contribute minimally to the spectrum. Identical, shallow melting curves were obtained for peptide **1** at concentrations of 5 µM and 245 µM, indicating that the folded form of peptide **1** is monomeric. Analysis of the spectrum in Figure 3 for secondary structure content[11b] failed to give reliable results, possibly because of spectral contributions derived from the side-chain bridges, or because of distortions in the helical structure that is present. A more detailed structural analysis of peptide **1** by ^{1}H-NMR will be required to confirm the extent and form of its α-helical structure.

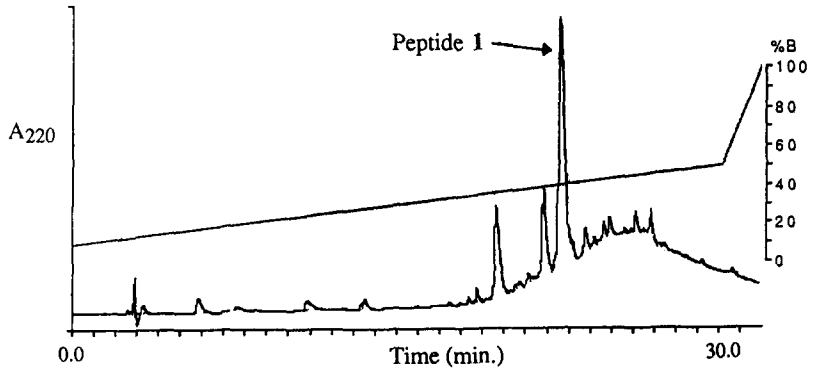


Figure 2. Reversed-Phase HPLC of Crude Peptide **1**: Peptide **1** was eluted on a C_{18} analytical column, using a buffer gradient of 10-50% buffer B in buffer A, at 1.2 mL/min. over 30 min., monitoring A_{220} (buffers: A = 0.1% TFA + 10% CH_3CN in H_2O; B = 0.1% TFA + 90% CH_3CN in H_2O). Peptide **1** eluted at 22.6 min..

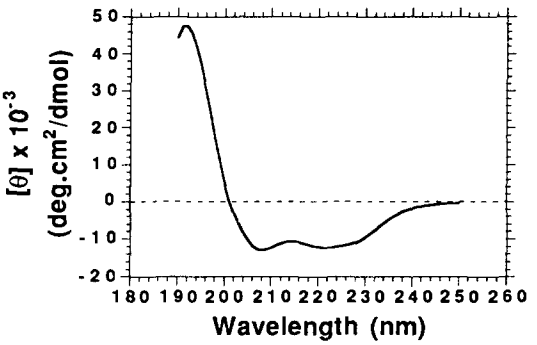


Figure 3. The CD spectrum of peptide **1** measured at 25°C in aqueous 10 mM sodium phosphate buffer, pH 7.0. Peptide **1** concentration was determined by amino-acid analysis with internal standards.

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