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Synthesis of a cyclic pseudopeptide containing a flexible β-Alaψ[CH_2NH]unit

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Abstract—The target cyclic pseudopeptide, cyclo(Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu), was efficiently synthesized using a methodology combining solid phase synthesis of the linear pseudopeptide and solution phase cyclization of the linear pseudopeptide.

Unnatural amino acids are useful building blocks for the study of structure–activity relationships of peptides, and in the design of peptide and peptidomimetic therapeutics.[1] β-Alanine, one of the commonly used unnatural amino acids, has attracted a lot of attention due to its unique flexible structure and ability to adopt either gauche or trans configurations within a restricted cyclic peptide host. Systematic analysis of β-alanine-containing cyclic peptides indicated that β-alanine residue possess a low propensity to be incorporated at the corner position of turn structures, but the presence of the flexible ethylene unit in β-alanine could reduce ring strain and possibly lead to new types of intramolecular hydrogen bonds.[2] Indeed, our recent study on a β-alanine-containing cyclic hexapeptide, cyclo(Tyr-Gly-β-Ala-Ala-Phe-Leu), by X-ray crystallographic methods reveals the presence of a novel eleven- membered turn structure encompassing '–Tyr-Gly-β-Ala-Ala–' residues.[3]

As a continuation of our previous study on the conformational behavior of cyclic pseudopeptides,[4] we elected to perform conformational analysis of β-alanine-containing cyclic peptides by incorporating a β-Alaψ[CH_2NH] unit into cyclic peptide hosts. We report herein the synthesis of one of our target pseudopeptides, cyclo(Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu).

The reduced peptide bond, ψ[CH_2NH], has been used previously in the design of ACE inhibitors,[5] and for the generation of potent antagonists of bombesin,[6] gastrin,[7] substance P[8] and secretin receptors,[9] to name a few. A general solid phase approach for the introduction of the ψ[CH_2NH] isostere was first reported by Sasaki and Coy.[10] The procedure consists of treating a Boc-protected amino aldehyde with a resin bound protonated amine in the presence of a reducing reagent, usually $NaBH_3CN$. However, this procedure failed to produce the desired β-Alaψ[CH_2NH] unit on the resin. Instead, a disubstituted product was generated, as shown in Scheme 1.

To solve this problem, we envisioned that two approaches could be potentially employed: (i) a resin-bound nucleophilic displacement reaction; and (ii) protection of the primary amine with a reversible protecting group before reductive amination (Scheme 2).

Indeed, our model studies indicated that both approaches worked and could be used for the introduction of the β-Alaψ[CH_2NH] unit into linear peptide. Subsequently, the linear precursor of the target compound, H-Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu-OH, was synthesized. As shown in Scheme 3, the β-Alaψ[CH_2NH] unit was successfully incorporated into the linear peptide by reacting a Boc-protected diaminopropane with the resin bound bromide. This displacement reaction proceeded in essentially quantitative yield.[11] The resulting secondary amine was then protected with a benzoxycarbonyl (–Z–) group, followed by the couplings of Boc-Gly-OH and Boc-Tyr(2,6-diClBzl)-OH successively to the solid support. The desired linear pseudopeptide,

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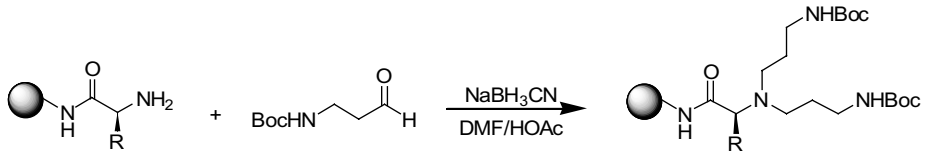
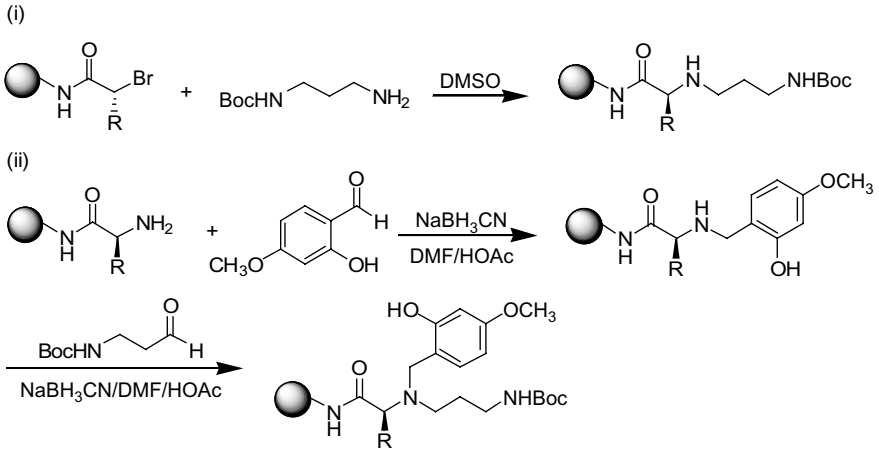
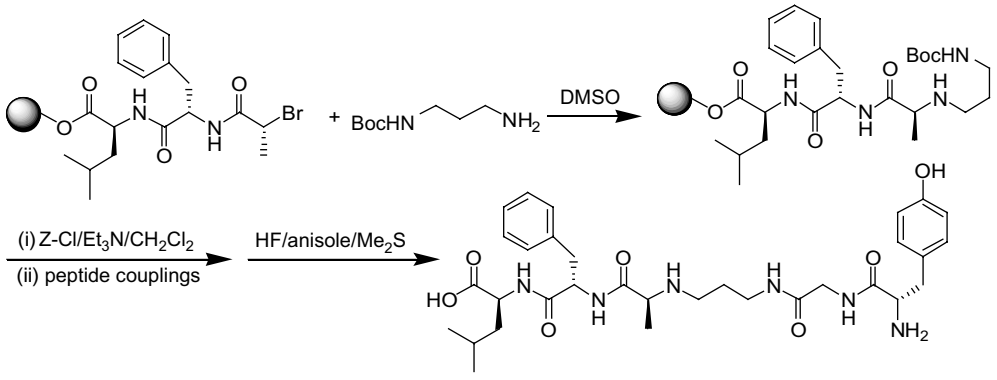


Scheme 1.



Scheme 2.

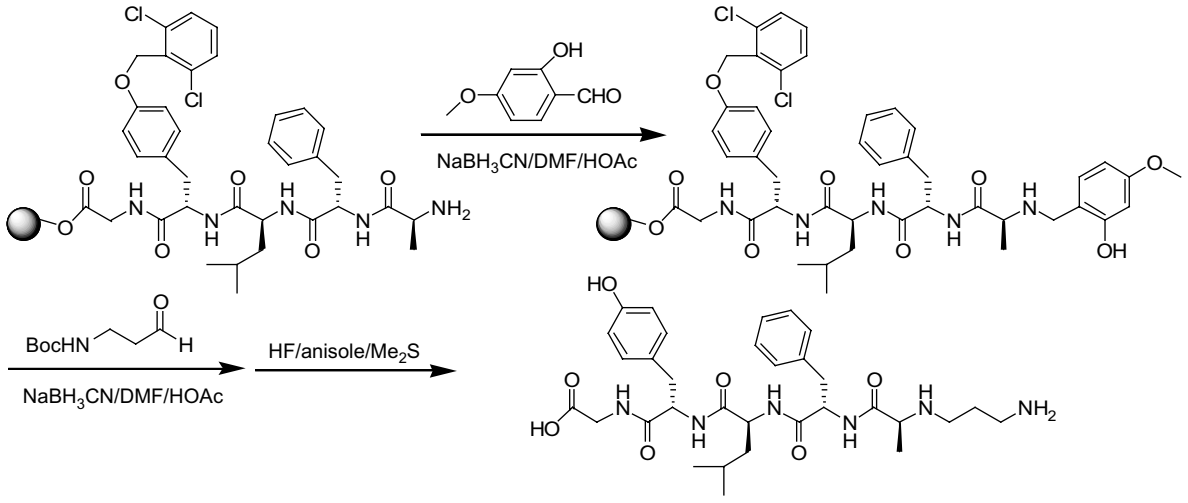


Scheme 3.

H-Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu-OH, was obtained after HF acidic cleavage. The crude product was further purified by an LH-20 column and cyclized with DPPA/$NaHCO_3$/HOBt.

Surprisingly, the cyclization process was very sluggish and did not proceed as expected. After three days, only about 15–20% of the linear pseudopeptide had been cyclized, as monitored by HPLC. The extremely low yield of this cyclization prompted us to look for a new synthetic alternative.

It is known that the cyclization rate of a linear peptide sequence is somehow unpredictable and may well be sequence dependent.[12] Since the linear pseudopeptide, H-Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu-OH, cyclized sluggishly in solution, we conjectured that a positional shift of the β-Alaψ[CH_2NH] unit within the linear pseudopeptide sequence might greatly facilitate the cyclization process. Based on our previous study on the cyclization rate of resin-bound linear pseudopeptide containing a ψ[CH_2NH] isostere,[13] we decided to put the β-Alaψ[CH_2NH] unit at the N-terminus of the linear pseudopeptide, and synthesized the linear pseudopeptide, H-β-Alaψ[CH_2NH]-Ala-Phe-Leu-Tyr-Gly-OH, accordingly (Scheme 4). The linear pseudopeptide thus obtained was purified and subjected to cyclization (DPPA/$NaHCO_3$/HOBt). As expected, the cyclization proceeded smoothly. The starting linear pseudopeptide disappeared completely from the cyclization mixture after three days, as judged by HPLC analysis. The desired cyclic pseudopeptide (cyclo[Tyr-Gly–β-Alaψ[CH_2NH]Ala-Phe-Leu), was finally purified to homogeneity by LH-20 column and semi-preparative HPLC, and characterized by ES-MS, ^{1}H NMR, AAA, and ^{13}C NMR.[14] The overall synthetic yield was 32%.



Scheme 4.

In conclusion, we have developed a practical approach for incorporating a β-Alaψ[CH_2NH] unit into a cyclic peptide host, and synthesized the desired cyclic pseudopeptide using a methodology combining solid phase synthesis of the linear pseudopeptide and solution phase cyclization of the linear pseudopeptide.

Acknowledgements

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References and notes

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11. Typical experimental procedure: 0.48 g Boc-NH–$(CH_2)_3$–NH_2 (2.7 mmol) in 3 mL of DMSO was added to a reaction vessel containing 0.27 mmol (D)-Br-Ala-Phe-Leu-resin. The reaction proceeded at room temperature for 4 h. The resin was washed with DMF, ethanol, and DCM, and dried under vacuum.
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14. cyclo[Tyr-Gly-β-Alaψ[CH_2NH]Ala-Phe-Leu], ^1H NMR (DMSO-d_6): δ (ppm) 8.63 (d, 1H), 8.47 (d, 3H), 7.75 (d, 1H), 7.70 (d, 1H), 7.68 (d, 1H), 4.41 (m, 1H), 4.30 (m, 1H), 4.17 (m, 2H), 4.12 (m, 1H), 3.66 (m, 1H), 3.62 (m, 1H), 3.32 (m, 1H), 3.14 (m, 1H), 3.02 (m, 1H), 2.96 (m, 1H), 2.93 (m, 1H), 2.83 (m, 1H), 2.75 (m, 1H), 1.74 (m, 1H), 1.66 (m, 1H), 1.50 (m, 1H), 1.46 (m, 4H), 1.41 (m, 1H), 0.82 (m, 6H); ^{13}C NMR (DMSO-d_6): δ (ppm) 55.3, 54.7, 53.9, 51.8, 42.4, 40.4, 36.2, 35.7, 35.4, 25.1, 23.6, 22.5, 20.7, 8.1; ES-MS: calcd for [$C_{32}H_{44}$–N_6O_6, H+] 609.7, found 609.5; Amino acid analysis: Tyr 0.94(1.00), Gly 1.03(1.00), Phe 0.98(1.00), Leu 0.96(1.00).