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Solid-phase synthesis of Mannich-base hybridized cyclopeptides

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Abstract—A synthesis of new cyclopeptides is reported via Mannich condensation on solid support with a simple work-up procedure and very good yields.

Resistance to enzymatic hydrolysis is critically important for the development of peptide-based drugs. The short half-life of unmodified peptides can be dramatically prolonged in vitro and in vivo by insertion of non-peptide structures into the peptide chain.[1–3] There is increasing interest in research on biologically active peptides with conformationally constrained structures, such as cyclopeptides. The goal is to obtain a more pharmacologically useful agent by eliminating undesirable properties (e.g. cleavage by proteases) while retaining affinity for the peptide's receptor. As far as cyclopeptides are concerned, those with unnatural bridged structures are usually more resistant to biodegradation than classical cyclopeptides with disulfide bonds or amide bonds as the bridge.

Herein, we report the successful application of Mannich-base insertion into a peptide backbone on solid support. The application of Mannich chemistry to resin bound substrates has been reported recently,[4–7] however, as far as we are aware the solid-phase synthesis of Mannich-base bridged cyclopeptides has not yet been documented. Generally, the Mannich condensation needs three components as the building blocks: an aldehyde, an amine and an active hydrogen component. In our protocol, the tyrosine residue, being easily anchored on solid support and joined at the C-terminal of the active peptide, served as the active hydrogen component, the N-terminal amino group as the amine component, and formaldehyde as the only component which was used in solution (Fig. 1). Firstly, tripeptidyl resin **3** as the substrate for subsequent cyclization was assembled by standard SPPS procedures, and then condensed with formalin giving cyclopeptidyl resin **4** (Scheme 1).

Formation of the thirteen-membered ring was completed after 5 days of reaction as monitored by a ninhydrin test.[8] The final product **5** was obtained in very good yield (overall 85%) and its structure was confirmed by FAB-MS. The success in the preparation of **5** spurred us to prepare other Mannich-base bridged cyclopeptides.

Initially, ring closure of tetrapeptidyl resin **7** with formaldehyde failed even under more drastic conditions (at 60–70°C for 10 days). We chose pentapeptidyl resin **8** with a secondary amine (Pro) at the N-terminus as the substrate for the Mannich condensation. By treatment similar to the ring closure of **3** and release of **5**, the nineteen-membered ring products **10** and **11** were

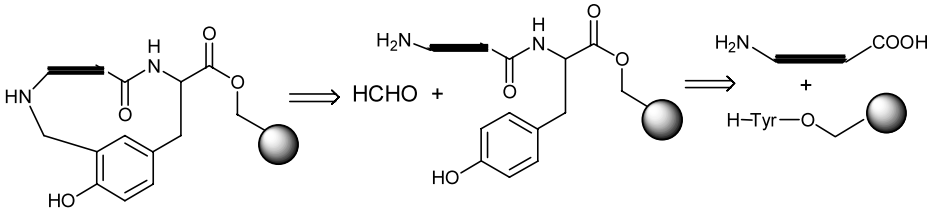

Figure 1. Retrosynthetic analysis for a Mannich-base hybridized cyclopeptide.

Keywords: Mannich condensation; cyclopeptide; solid-phase synthesis.

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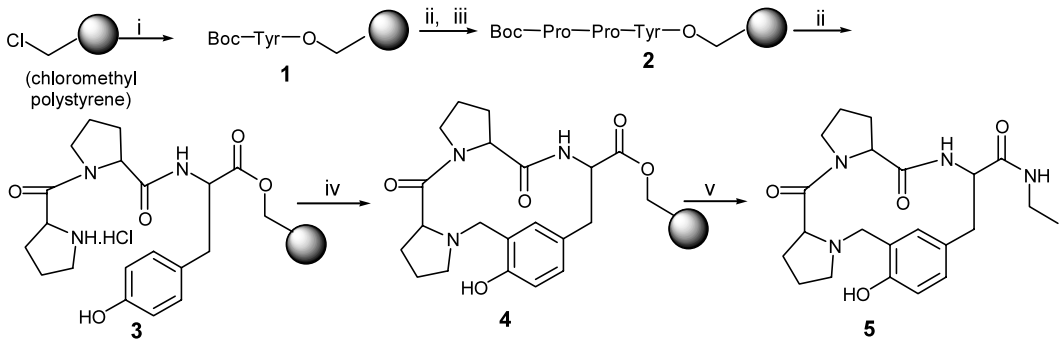
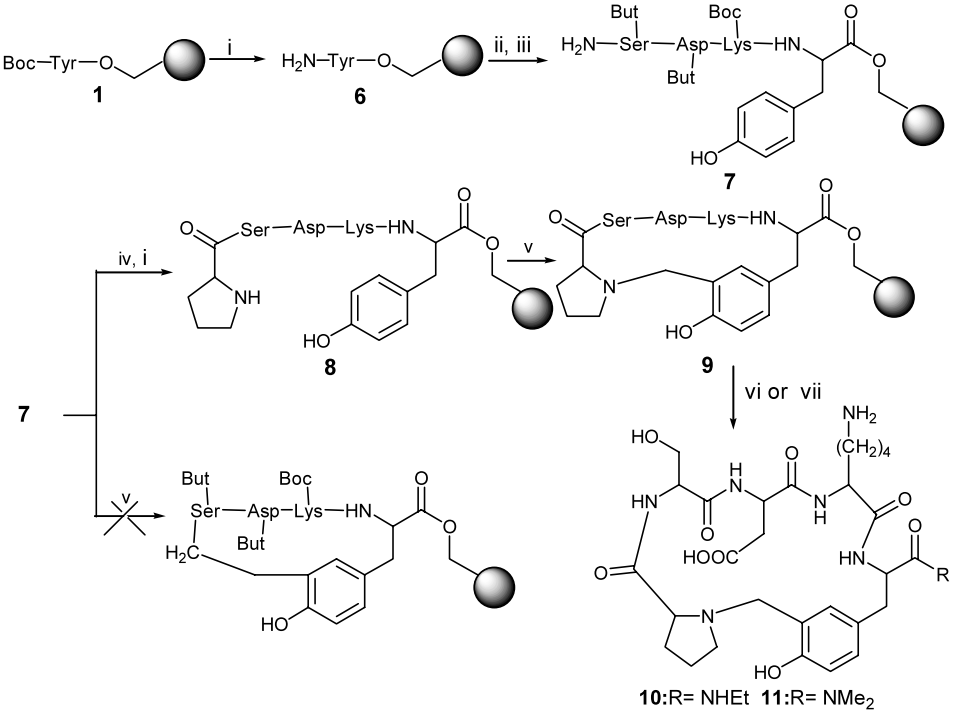


Scheme 1. *Reagents and conditions*: (i) Boc-Tyr-OH, Cs_2CO_3, NaI (cat.)/DMF; (ii) 50% TFA/DCM; (iii) Boc-Pro-OH, DCC, HOBt, NMM/DMF; (iv) 37% HCHO/H_2O–THF (3:7); (v) 70% $EtNH_2$/H_2O–THF (1:1).



Scheme 2. *Reagents and conditions*: (i) a. 50% TFA/DCM, b. 6% TEA/DCM; (ii) Fmoc-Aa-OH, DCC, HOBt/DMF; (iii) 20% piperidine/DMF; (iv) Boc-Pro-OH, DCC, HOBt/DMF; (v) 37% HCHO/H_2O–THF–concd. HCl (30:69:1); (vi) 70% $EtNH_2$/H_2O–THF (1:1); (vii) 33% $HNMe_2$/H_2O–THF (1:1).

obtained in overall yields of 81 and 78%, respectively (Scheme 2).

Since pentapeptidyl resin **8** contains a Ser-Asp-Lys fragment, another pentapeptidyl resin **12** containing a Lys-Asp-Ser fragment was also treated with formaldehyde to check the influence of the amino acid sequence upon the Mannich cyclization on solid support. Using the same protocol as in Scheme 2, the targeted compound **13** (with a nineteen-membered ring) was produced, also in good yield (Scheme 3). In summary, we have described an efficient protocol for the solid-phase preparation of Mannich-base hybridized cyclopeptides using proline and tyrosine as the amine and the active hydrogen component respectively. The very good yields of all products by this protocol indicated the Mannich-type cyclization on solid support was independent both of the amino acid sequence and of the length of the peptide chain. Moreover, the pseudo-dilution effect concerned with solid support should be responsible for the intramolecular condensation thereby avoiding the intermolecular reaction. The pertinent results are summarized in Table 1.

Acknowledgements

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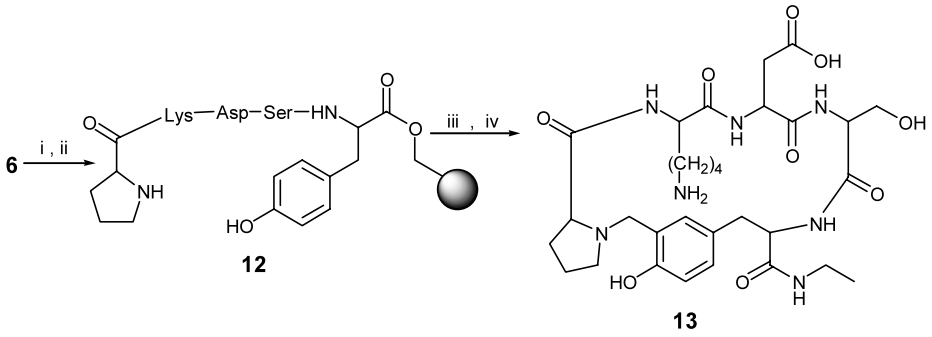

Scheme 3. *Reagents and conditions*: (i) Fmoc-Aa-OH, DCC, HOBt/DMF; (ii) 20% piperidine/DMF; (iii) 37% HCHO/H_2O–THF–concd. HCl (30:69:1); (iv) 70% $EtNH_2/H_2O$–THF (1:1).

Table 1. The results of the preparation of Mannich-base hybridized cyclopeptides

	Structure of products	Yield(%)	AAA[a]	FAB-MS (M+1)
5	Pro-Pro-Tyr-NHEt └CH_2┘	85	Pro 2.13(2), Tyr 0.98(1)	417.2
10	Pro-Ser-Asp-Lys-Tyr-NHEt └—— CH_2 ——┘	81	Asp1.01(1), Ser 0.95(1), Tyr 0.96(1), Lys1.07(1), Pro 1.06(1)	648.3
11	Pro-Ser-Asp-Lys-Tyr-NMe_2 └—— CH_2 ——┘	78	Asp1.01(1), Ser 0.95(1), Tyr 0.96(1), Lys1.07(1), Pro 1.06(1)	648.2
13	Pro-Lys-Asp-Ser-Tyr-NHEt └—— CH_2 ——┘	80	Asp1.02(1), Ser 0.96(1), Tyr0.95(1), Lys 1.06(1), Pro 1.05(1)	648.3

[a] Automated Amino acid Analyses

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8. (a) **General procedure for the Mannich-type cyclization**. The cyclization reaction was performed by mixing the deprotected peptidyl resin (2 mmol) with 20 mL of 37% HCHO/H_2O–THF (3:7) (for **4**)–or 37% HCHO/H_2O–THF–concd. HCl (30:69:1 for **9** and **13**). The reaction mixture was shaken in a sealed tube at 60°C for 5 days (the conversion of the imino group of proline was monitored by the ninhydrin test). The supernatant was drained off. The remaining resin was washed successively with MeOH (×3), DMF (×2), MeOH (×5) and Et_2O (×2).
(b) **General procedure for releasing the final products by aminolysis**. The cyclopeptidyl resin (2 mmol) and 20 mL of 70% $EtNH_2/H_2O$–THF (1:1) (for **5**, **10** and **13**) or 33% $HNMe_2/H_2O$–THF (1:1) (for **11**) were mixed in a sealed tube. The reaction mixture was shaken at rt for 24 h. The supernatant was collected and concentrated in vacuum to dryness. To the residue dry ether (30 mL) was added. After triturating, the powdered product was collected by filtration. The four products were all characterized by AAA and MS (Table 1).