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Solid-Phase Synthesis of 1,2,3,4-Tetrahydro-β-carboline-Containing Peptidomimetics

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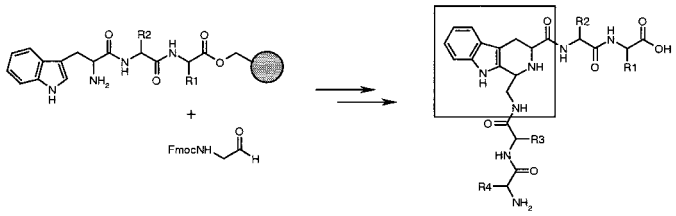
ABSTRACT



A solid-phase method for the synthesis of 1,2,3,4-tetrahydro-β-carboline-containing peptidomimetics has been developed. The key step in the strategy is the Pictet–Spengler condensation of a resin-bound tryptophan-containing fragment with an Fmoc-amino aldehyde.

Solid-phase organic synthesis is a powerful tool for the rapid generation of large numbers of compounds for the investigation of the effects of structure on biological activity.[1,2] This technology, which originated with Merrifield peptide synthesis,[3] has recently been widely used for the synthesis of small organic molecules and non-peptide peptidomimetics. In the search for new peptide bond replacements, one approach has been the construction of a nonpeptidic central scaffold for displaying pharmacophoric elements[4] or incorporating heterocyclic compounds in a peptide sequence.[5] These manipulations also introduce conformational constraints to the structure that enhance activity and alter the structure−activity relationships.[6]

Various constrained tryptophan analogues or tryptophan-containing motifs have been utilized to generate highly potent and selective ligands to biological target receptors. Representative examples include analogues of somatostatin,[7a] cholecystokinin,[7b] melanotropin[7c] and growth hormone

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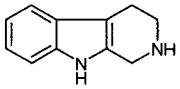
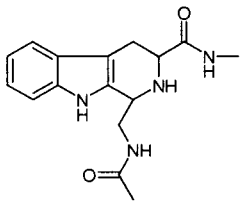
secretagogues.[7d] The 1,2,3,4-tetrahydro-β-carboline (THBC) core **1** is a common feature of many tryptophan-derived indole and isoquinoline alkaloids (Scheme 1).[8] Natural and

Scheme 1. 1,2,3,4-Tetrahydro-β-carboline and THBC-Containing Peptidomimetic Bond



1. 1,2,3,4-tetrahydro-β-carboline (THBC)



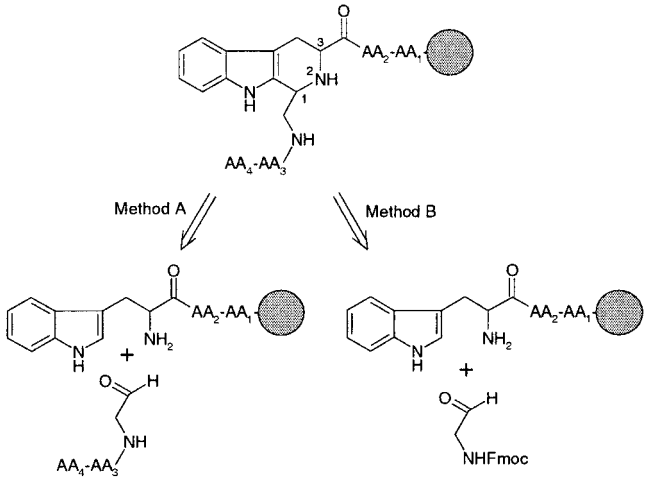
2. THBC-containing peptidomimetic bond

synthetic products containing a β-carboline pharmacophore exhibit a wide range of important medicinal properties. They are known to bind with high affinity to benzodiazepine[8] and serotonin[9] receptor sites. Therefore, the rigid and heterocyclic nucleus of the THBC-containing moiety **2** provides a promising scaffold for the design of pharmacophore-based libraries of THBC-containing peptidomimetics. In this Letter, we report a solid-phase method for the synthesis of this class of compounds.

Traditionally THBC derivatives have been prepared by the Pictet−Spengler condensation of tryptophan with aliphatic or aromatic aldehydes.[10,11] Recently we found that peptide aldehydes are also good substrates for this acid-catalyzed condensation.[12] The reaction of a peptide C-terminal aldehyde with N-terminal tryptophan-containing fragments in acetic acid afforded the corresponding ligation products with the THBC linkage. Extending the application of this reaction to solid support would provide a convenient method for generating THBC-containing peptidomimetics.

We chose commercially available Fmoc-amino acid Wang resin (Advanced ChemTech, Louisville, KY) as the solid support. Wang resin has been extensively utilized for solid-phase synthesis and is stable to TFA in low concentration (<5%).[13] Our initial experiments focused on a modular approach that uses *N*-Fmoc peptide aldehydes as starting materials[12a] for a condensation reaction with resin-bound tryptophan (method A, Scheme 2). This approach produced

Scheme 2. Methods To Synthesize Peptidomimetics on Resin



the desired THBC-containing compounds on solid support in a single step. Subsequent chemical transformation steps were omitted to avoid potential side reactions with the newly formed secondary nitrogen (nitrogen 2) in the THBC structure. As a representative example, Fmoc-Leu-Leu-Ala-Gly-H was allowed to react with Trp-Leu-Ala-Wang resin for 24 h in 1% TFA in dichloromethane, and the THBC-containing heterocycle was formed on the resin. After Fmoc removal, the desired product **7a** was obtained in high yield and 90% purity after TFA cleavage (Table 1, entry 1).

During our exploratory studies, we found that the nitrogen at position 2 of the THBC core remained unreacted under standard coupling conditions of Fmoc amino acids (DIC/HOBt/DMF).[14] Lack of reactivity of that nitrogen is presumably due to steric hindrance. This phenomenon has also been observed in other THBC derivatives derived from organic aldehydes.[11a,b,f] These results prompted us to investigate a stepwise strategy (method B, Scheme 2) in which an Fmoc amino aldehyde was used in the Pictet−Spengler condensation step followed by peptidomimetic chain assembly on the same resin.

Scheme 3 shows the solid-phase synthesis of THBC-containing peptidomimetics. The Fmoc group was first

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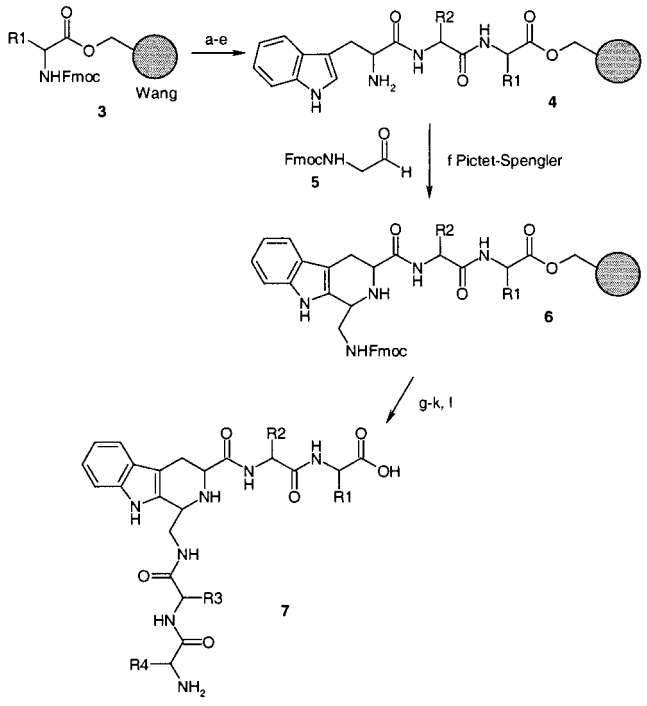
Table 1. Representative THBC-Containing Peptidomimetics

entry	R_1-R_2 (from parent amino acids)	R_3-R_4 (from parent amino acids)	purity[a] (%)
7a[b]	Ala-Leu	Ala-Leu-Leu	90
7b	Ala-Phe	Pro-Val	87
7c	Ala-Ala	Phe-Leu	86
7d	Ala-Phe	Asp-Gly	85
7e	Ala-Ala	Lys-Ala	90
7f	Phe-Leu	Ala-Gly	85
7g	Phe-Val	Leu-D-Phe	94
7h	Phe-Leu	Asp-Gly	81
7i	Phe-Val	Lys-Ala	80
7j[c]			95
7k	Ala-Leu		90
7l	Ala-Leu	Phe	90
7m	Ala-Cha[d]	Phg[e]-PyAla[f]	89
7n	Ala-Cha	Phg-Lys	78
7o	Ala-Cha	Phg-D-Phe	87

[a] Purity was based on HPLC. [b] The synthesis was carried out by method A using Fmoc-Leu-Leu-Ala-Gly-H. [c] The synthesis was carried out using Fmoc-Gly-H with Trp-Wang resin. [d] β-Cyclohexylalanine. [e] Phenylglycine. [f] 2-L-Pyridylalanine.

removed from Fmoc-amino acid (AA_1) Wang resin **3**, and the second amino acid (AA_2) was attached to the resin using standard coupling conditions. Subsequently, Fmoc-Trp was introduced to the solid support without any side chain protection. After removal of the Fmoc group, the resin **4** contained a free N-terminal tryptophan.

Scheme 3. Solid-Phase Synthesis of THBC-Containing Peptidomimetics on Wang Resin[a]



[a] Reagents and conditions: (a) 20% piperidine/DMF; (b) Fmoc-AA_2/DIC/HOBt; (c) 20% piperidine/DMF; (d) Fmoc-Trp/DIC/HOBt; (e) 20% piperidine/DMF; (f) Fmoc-Gly-H, 1% TFA/DCM, 24 h; (g) 20% piperidine/DMF; (h) Fmoc-AA_3/DIC/HOBt; (i) 20% piperidine/DMF; (j) Fmoc-AA_4/DIC/HOBt; (k) 20% piperidine/DMF; (l) 95% TFA.

The key step in our stepwise strategy is a condensation between the resin-bound tryptophan **4** and an amino aldehyde, Fmoc glycinal **5**.[15] The resin-bound free amine **4** was allowed to react with a 10 molar equiv excess of aldehyde **5** in 1% TFA in dichloromethane at room temperature.[16] A solution of 1% TFA was found to be sufficient for this acid-catalyzed condensation without causing cleavage of the product from the resin. The Pictet−Spengler condensation of the resin-bound tryptophan **4** with Fmoc-glycinal **5** could be conveniently monitored by the Kaiser ninhydrin test, and a completion of the condensation step was ensured when a negative test was observed.[17] Generally the reaction was completed in 12 h. Formation of the condensation product was confirmed by TFA cleavage from the resin **6** and followed by HPLC[18] and LC-MS analysis.[19] In RP-HPLC, unreacted Trp-containing product eluted much earlier, compared to the heterocyclic product containing a hydrophobic Fmoc group. The formation of the THBC heterocyclic structure was further confirmed by ^{1}H NMR analysis of compound **7j**, which was prepared by the Pictet−Spengler reaction of Fmoc glycinal with Trp-Wang resin under similar reaction conditions.[20]

When the condensation was complete, the resulting resin-bound THBC heterocycle **6** was deprotected with 20% piperidine in DMF. Elongation of the peptidomimetic backbone (AA_3, AA_4) was performed with a 5 molar equiv excess of Fmoc amino acids (DIC/HOBt/DMF), followed by piperidine deprotection. The final product **7** was cleaved from the solid support by TFA treatment. Representative examples, THBC-containing products (Table 1), were obtained in essentially quantitative yields, with a purity level typically in excess of 80%. The product peaks eluted from HPLC corresponded to the expected molecular masses.[21] In our studies, no double acylation products were detected by

(15) Fmoc-glycinal **5** was prepared by the reaction of aminoacetalaldehyde dimethyl acetal with Fmoc-Cl, followed by deprotection with 30% TFA in CH_2Cl_2 according to the following: Liu, C.-F.; Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6584−6588.

(16) **Procedure for the Pictet−Spengler condensation:** To a suspension of the resin **4** (100 mg, 0.74 mmol/g) in 1% TFA/CH_2Cl_2 (2 mL) was added a 10 molar equiv excess of Fmoc-glycinal. After the mixture was allowed to react at room temperature overnight, the resin was washed with DMF (3 × 2 mL) and CH_2Cl_2 (3 × 2 mL). If the ninhydrin test showed a positive result, then the procedure was repeated. The resin was used directly for the next step of the reaction. Final products were cleaved from the resin with TFA/water (95:5).

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(18) HPLC was performed on a Vydac C_{18} column (250 × 4.6 mm) at a flow rate of 1 mL/min (buffer A, 0.05% TFA in H_2O; buffer B, 0.05% TFA in 60% CH_3CN in H_2O). Gradient: 10% to 100% B within 15 min. UV monitoring at 220 nm.

(19) LC-MS was performed on a Hewlett-Packard 1100 system.

(20) ^{1}H NMR for compound **7j** (DMSO-d_6, 300 MHz), δ 11.1, 11.2 (s, 1H), 7.0−7.6 (m, 4H), 4.7−4.9 (m, 1H), 4.1−4.3 (m, 1H), 3.2−3.4 (m, 2H), 2.9−3.2 (m, 2H).

(21) In most cases the diastereomeric isomers **7** of these THBC-containing compounds coeluted under the HPLC conditions. ^{1}H NMR analysis of the THBC core **7j** indicated that the diastereomeric ratio is approximately 1:1 in the mixture.

LC-MS analysis. Several unnatural and hindered amino acids such as Cha and Phg were also incorporated in the structure without difficulties.

In summary, we have developed an efficient solid-phase synthesis of THBC-containing peptidomimetics. The key reaction, formation of β-carboline heterocycles, is carried out on solid support at room temperature under mild reaction conditions. In this procedure, the desired peptidomimetics are formed using readily available building blocks, natural or unnatural amino acids, and an Fmoc-amino aldehyde. The incorporation of heterocyclic THBC into the peptide sequence is also expected to generate conformational constraints and potential turn mimetics. The ready accessibility of other Fmoc-amino aldehydes[22] should allow the introduction of additional molecular diversity to the structure. The synthesis is also compatible with current methods of automation and can provide large libraries of spatially distinct molecules for biological assays.

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