ORGANIC LETTERS

2004
Vol. 6, No. 23
4239−4241

A New Strategy To Produce β-Peptides: Use of Alicyclic β-Lactams

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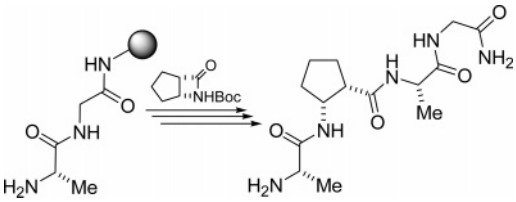
Received August 18, 2004

ABSTRACT



On *p*-methylbenzhydrylamine (MBHA) resin, by means of *t*-Boc chemistry, several tetrapeptides (H-Ala-ACXC-Ala-Gly-NH_2) containing cyclic β-amino acid units were prepared. These units were introduced into the growing peptide chain by using Boc-protected β-lactams with KCN as catalyst in DMF. The method was applicable for both racemic and enantiomeric β-lactams.

In 1989, two groups independently isolated an antifungal antibiotic, cispentacin, (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid.[1,2] Following the discovery of cispentacin, considerable attention was paid to alicyclic β-amino acids.[3,4] Although β-amino acids have been widely used for β-peptide synthesis,[5,6] the first incorporations of cyclic β-amino acids into peptides were described only a decade ago.[7] Following the pioneering work of Seebach et al.[8] on acyclic β-amino acids and peptides, a real breakthrough in the use of cyclic β-amino acids started several years later, when Gellman and coworkers proved that *trans*-aminocycloalkanecarboxylic acid peptide oligomers can fold into stable helical structures.[9,10] Our group recently demonstrated that the foldameric structure can be controlled: cispentacin oligomers adopt a sheet-type structure.[11] Insertion of a cyclic β-amino acid into the peptide chain can modify and improve the pharmacological activity

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10.1021/ol048356t CCC: $27.50
Published on Web 10/14/2004

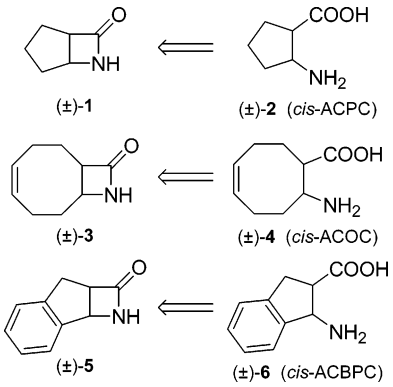
and increase the peptide stability, and this has therefore led to the formation of a number of such products.[12,13]

Various enantioselective methods are known for the synthesis of β-lactams.[14−16] Enantioselective methods or kinetic resolutions lead to enantiomeric β-lactams.[17] Since nucleophilic attack opens the ring without difficulty, these compounds can be used as β-amino acid precursors.[4,18] When an amine is used as nucleophile, the product is the corresponding amide.[19] The strategy is generally referred to as the β-lactam synthon method.[20] Palomo and co-workers utilized this synthetic strategy in the liquid phase for the synthesis of short peptide segments.[21] Our present aim was to extend the peptide synthetic strategies to cyclic β-peptides and to use diverse cyclic β-lactams in peptide syntheses, on a solid support.

A variety of model cyclic β-lactams were selected: a five-membered ring (±)-**1** (a racemic cispentacin precursor), an eight-membered ring with a double bond, (±)-**3**, and a benzene-fused five-membered system (±)-**5** (indan system), Scheme 1. The syntheses of the starting model compounds were straightforward: 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to cyclopentene, 1,5-cyclooctadiene, or indene, respectively, resulting in the corresponding β-lactams,[22] which after *tert*-butoxypyrocarbonate treatment afford the corresponding racemic *N*-Boc β-lactams. The enantiomeric *N*-Boc-protected (1*S*,5*R*)-6-azabicyclo[3.2.0]-heptan-7-one was prepared by lipolase-catalyzed kinetic resolution of (±)-6-azabicyclo[3.2.0]heptan-7-one, followed by *N*-Boc protection.[17a]

The peptide sequences **10**−**13** (H-Ala-ACXC)-Ala-Gly-NH_2) were synthesized by a solid-phase technique, utilizing

Scheme 1. Cyclic β-Lactams Used as β-Amino Acid Precursors



t-Boc chemistry.[23] The peptide chains were elongated on an MBHA resin, and the syntheses were carried out manually. Couplings of Gly and Ala were first performed with dicyclohexylcarbodiimide (DCC) without difficulty. After the incorporation of Ala, the Boc-protected β-lactams were introduced into the growing peptide chain, with KCN as catalyst in DMF. In the last step, Ala was again inserted. Amino acid incorporation was monitored by the ninhydrin test.[24] The completed peptide resins were treated with liquid HF/dimethyl sulfide/*p*-cresol/*p*-thiocresol at 0 °C for 1 h. HF was removed, and the resulting free peptides were solubilized in 10% aqueous acetic acid, filtered, and lyophilized. The crude peptides were investigated and purified by reversed-phase HPLC. The model peptides were characterized by mass spectrometry, using a tandem quadrupole mass spectrometer equipped with an electrospray ion source.

Scheme 2 shows the synthetic pathway when, for example, (1*S*,5*R*)-6-azabicyclo[3.2.0]heptan-7-one was used for the synthesis of tetrapeptide **10**. The HPLC analysis indicated that no epimerization occurred during the synthesis. When racemic β-lactams **1**, **3,** and **5** were used, the HPLC profiles showed two separate tetrapeptide signals with the same molecular mass. The peaks were easily separated, except in the case of (±)-6-azabicyclo[3.2.0]heptan-7-one, where the diastereomeric peaks for the tetrapeptide partly overlapped.

The model tetrapeptides (Table 1) synthesized (H-Ala-ACXC)-Ala-Gly-NH_2**)** were as follows: **10**, H-Ala-(1*S*,2*R*-

Table 1. HPLC and MS Characterization of the Synthetized Model Peptides[a]

code	calcd *m*	found *m*	HPLC t_R (**A**)	HPLC t_R (**B**)
10	327.39	328	6.14[b]	
11	327.39	328	6.12[b]	6.44[b]
12	367.75	368	12.70[c]	17.22[c]
13	375.43	376	14.33[d]	15.33[d]

[a] HPLC column: Vydac HS 201 (4.6 × 250 mm). The solvent system used was 0.1% trifluoroacetic acid (TFA) in water, 0.1% TFA, 80% acetonitrile in water, gradients: [b] 5% → 80% in 25 min, flow 1.2 mL/min, detection at 220 nm. [c] 15% → 30% in 30 min, flow 1 mL/min, detection at 220 nm. [d] 0% → 60% in 20 min, flow 1 mL/min, detection at 220 nm.

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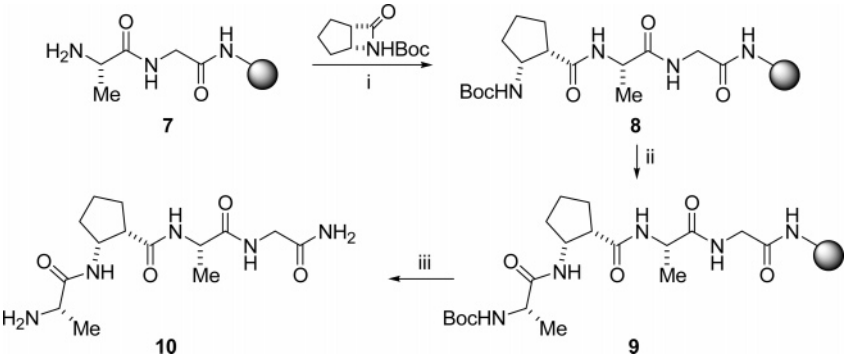
Scheme 2. Synthesis of Peptide **10**[a]



[a] Reagents and conditions: (i) 3 equiv of Boc-β-lactam, 3 equiv of KCN, DMF, 40 °C, 24 h; (ii) 3 equiv of Boc-Ala, 3 equiv of DCC, 3 equiv of HOBt, DMF, 24 °C, 2 h; (iii) liquid HF/dimethyl sulfide/*p*-cresol/*p*-thiocresol (86:6:4:2), 0 °C, 1 h.

ACPC)-Ala-Gly-NH_2 (1*S*,2*R*-ACPC) = (2S,2*R*)-2-aminocyclopentanecarboxylic acid); **11**, H-Ala-ACPC-Ala-Gly-NH_2 (ACPC = *cis*-2-aminocyclopentanecarboxylic acid); **12**, H-Ala-ACOC-Ala-Gly-NH_2 (ACOC = *cis*-2-amino-5-cyclooctenecarboxylic acid); and **13**, H-Ala-ACBPC-Ala-Gly-NH_2 (ACBPC = *cis*-1-aminoindane-2-carboxylic acid).

In conclusion, a simple and efficient direct method was developed for the introduction of β-peptide elements into a peptide. On a *p*-methylbenzhydrylamine resin, by means of *t*-Boc chemistry, a variety of cyclic β-amino acids were inserted into a peptide chain. These units were introduced into the growing peptide chain by using Boc-protected β-lactams, with KCN as catalyst in DMF. The method is applicable for both racemic and enantiomeric β-lactams. The diastereomers formed were in most cases easily separable. Since a number of lactams are readily available in racemic or enantiomerically pure form, the present method seems to offer a widely applicable alternative route for β-peptides and for combinatorial peptide libraries.

Acknowledgment. We acknowledge receipt of OTKA grants T 034901, T 034912, and TS 040888.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048356T

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