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An efficient approach for solid-phase synthesis of peptidomimetics based on 4-imidazolidinones

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Abstract—An efficient method for the incorporation of an imidazolidinone moiety into peptide sequences via an alkylidene bridge between two neighboring amide bonds is presented. A benzotriazole-mediated reaction of aldehydes with resin-bound peptides provides *N*-terminal 4-imidazolidinone-peptide derivatives. The generated five-membered ring, which will be expected to have similar properties to 'pseudo-prolines', can be acylated at the secondary amine to allow elongation of the peptide backbone. NMR studies of imidazolidinone-containing peptides show the existence of *cis*/*trans* isomerization, typical for proline-based peptides.

Peptidomimetics involving specific amide conformational constraints may mimic important structural features of pharmaceutically interesting peptides and simultaneously maintain beneficial biological activities. Modification of an amide backbone is often necessary for enhancing enzymatic stability of a peptide lead in drug research.[1] An alternative use of pseudo-prolines focuses on a temporary transformation of physicochemical properties of peptide sequences.[2] This concept was initially introduced by Mutter in order to prevent peptide aggregation and improve solubility with a minimum modification of backbone and/or functional groups.[2] Pseudo-proline derivatives have been constructed as reversible protecting groups of certain amino acids (Ser, Thr and Cys) that can be revealed under defined conditions. This approach has a number of potential advantages: (i) it simplifies the synthesis of peptides that tend to form highly aggregating complexes; (ii) it temporarily masks an active sequence, which can be released under defined conditions, and (iii) the constraint itself may enhance or modify both physical and physiological properties of the peptide. Herein, we present a novel concept of incorporation of 4-imidazolidinone moiety into the peptide sequence in order to examine the above mentioned benefits.

Until now, synthetic approaches for the preparation of 4-imidazolidinones have been exclusively based on solution chemistry strategies. Typically, the starting material is a protected amino acid or dipeptide that undergoes intramolecular cyclization with an aldehyde. The reaction can be catalyzed by acids[3] or bases.[4] No catalyst is needed with acetone.[5] Other methods involve the Beckmann rearrangement[6] of azetidin-3-one, condensation of α-aminonitriles with carbonyl compounds,[7] or anodic oxidation of protected dipeptide esters.[8] *N*-terminal 4-imidazolidinone formation was originally introduced as a temporary protecting group.[5,9] There are also examples of spontaneous formation of this *N*-terminal modification of peptides or proteins with either acetaldehyde under physiological conditions resulting from alcohol intoxication[10] or as a side reaction with formaldehyde or acetone.[11] 4-Imidazolidinones derived from aromatic amino acids and acetone have been used as moderately active inhibitors of tyrosine and histidine decarboxylase.[12] Dipeptide-based isopropylidene-4-imidazolidinones have been found to aid memory loss associated with aging.[13] A concept of peptide prodrugs with *N*-terminal temporary protection via imidazolidinone formation has also been examined on a set of diverse analogs of Leu-Enkephalin.[14]

Recently, we have developed a solid-phase method for the preparation of 1,2,5-trisubstituted 4-imidazolidinones[15] enabling either parallel or combinatorial synthesis via Katritzky's benzotriazole-approach.[16] The reaction is based on the formation of an *N*-[1-(benzotriazol-1-yl)alkyl] intermediate that undergoes a spontaneous nucleophilic substitution. Here we report the

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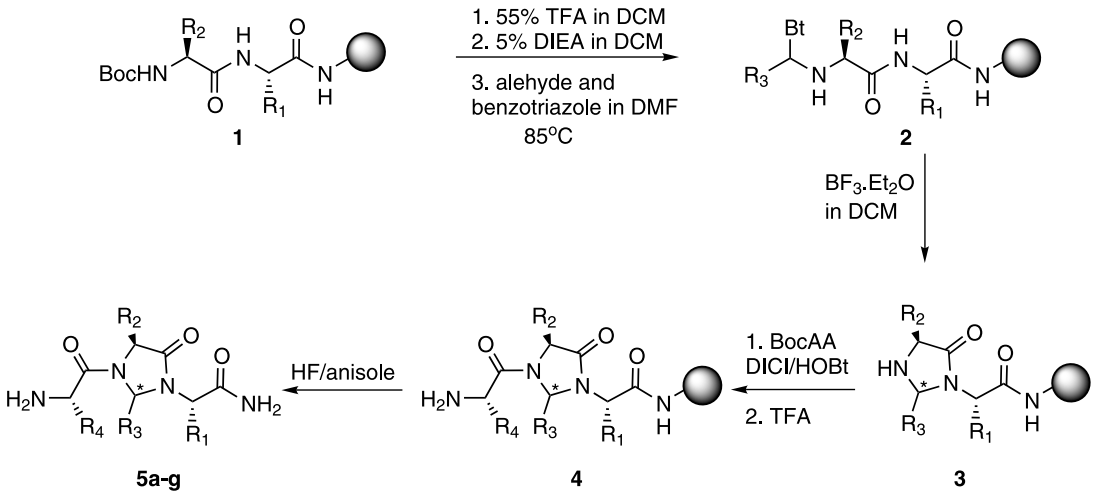
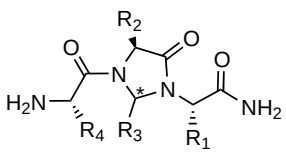
utilization of a similar approach enabling incorporation of a 4-imidazolidinone grouping into a peptide sequence, in order to constrict the peptide conformation through an alkylidene bridge between two neighboring amides.

The parallel synthesis of tripeptidomimetics was carried out using the solid-phase 'tea-bag' methodology (Scheme 1).[17] The starting resin-bound *N*-tert-butyloxycarbonyl (Boc) protected dipeptide sequences **1** were prepared using standard solid-phase peptide synthesis.[18] Following deprotection of the Boc group using 55% trifluoroacetic acid (TFA) in dichloromethane (DCM), neutralization with 5% *N*,*N*′-diisopropylethylamine (DIEA), and washing with DCM, the resins were treated with formaldehyde or butyraldehyde in a presence of benzotriazole (BtH) to yield *N*-[1-(benzotriazol-1-yl)alkyl] intermediates **2**.[19] The cyclization step to the imidazolidinone **3**, was accomplished by treatment of **2** with $BF_3{\cdot}Et_2O$.[20] The resin was then thoroughly washed with DCM, MeOH, toluene and DMF. The generated secondary amine **3**, was coupled to a Boc-protected amino acid (10 equiv.) in the presence of an equimolar amount of diisopropylcarbodiimide (DICI) and *N*-hydroxybenzotriazole (HOBt) in *N*,*N*′-dimethylformamide (DMF). Following deprotection of the terminal Boc group, the resin was cleaved with HF (1.5 h, 0°C). Following extraction and lyophilization, the desired products **5** were obtained as white or yellowish solids in almost quantitative yields. All the products were characterized by LC-MS, RP HPLC and ^{13}C NMR.[21] The results are summarized in Table 1. Generally, the ring formation is not stereoselective; a new chiral center is formed at the 2-position of the imidazolidinone (with the exception of formaldehyde). Attempts to prepare analogous compounds using ace-



Scheme 1.

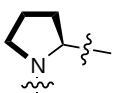
Table 1. 4-Imidazolidinone tripeptidomimetics



Comp. No.	R_1	R_2	R_3	R_4	MW expect./found	Yield[a] (%)	Purity[b] (%)
5a	$PhCH_2$	Me	H	4-OH-$C_6H_4CH_2$	410.2/410.8	92	82
5b	$PhCH_2$	Me	Pr	4-OH-$C_6H_4CH_2$	452.2/453.4	85	85
5c	*i*Pr	H	H	$PhCH_2$	332.2/333.2	81	73
5d	$PhCH_2$	H	H	*i*Bu	346.2/347.2	89	65
5e	$NH_2(CH_2)_4$	$CH_3CH(OH)$	Pr	$PhCH_2$	447.3/448.2	84	78
5f	$HOOC(CH_2)_2$	4-OH-$C_6H_4CH_2$	Pr	$HOOC(CH_2)_2$	492.2/493.3	89	70
5g	$HOOC(CH_2)_2$	$HOOC(CH_2)_2$	Pr	-$(CH_2)_3$-[c]	426.2/427.1	97	53

[a] Yield of crude product based on resin substitution.

[b] Purity is based on the integration of HPLC traces at 214 nm.

[c] -$(CH_2)_3$- : 

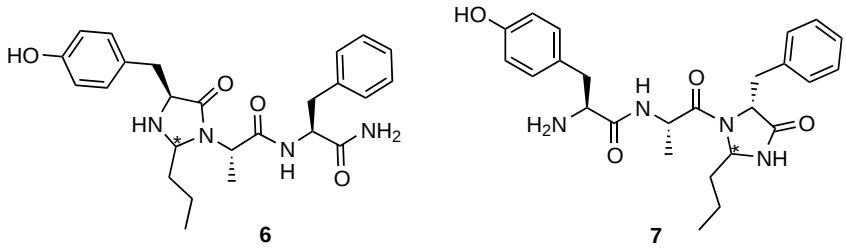


Figure 1.

tone failed. In this case, the synthesis was terminated at intermediate **3**, which could not be acylated, most likely due to the steric hindrance of the two methyl groups at the 2-position. We have also investigated a number of aromatic aldehydes without obtaining the cyclic product. The only product, in this case was the tripeptide. Because we have already shown that intermediate **3** can form,[15b] it seems probable that the imidazolidinone **4** formed having an aryl group in the 2-position was not stable in HF.

Analysis of products was complicated by the presence of two diastereomers in each case (except for those products with a methylene bridge derived from formaldehyde). The tertiary amide bond formation is represented by the presence of two distinctive *cis* and *trans* rotameric forms for each diastereomer which results in broader or split peaks provided by standard HPLC and double signals in NMR for atoms surrounding or participating on these amide groupings. This behavior can be eliminated by using higher temperature during measurement.

In the course of incorporation of an alkylidene bridge into the peptide sequences, we have prepared in a similar manner analogs **6** and **7** (Fig. 1). Compound **6** was prepared by cyclization of a resin bound tripeptide (H-Tyr(*O*-benzyl)-Ala-Phe-resin) with butyraldehyde. The resulting product[22] was obtained following HF treatment in 75% yield together with some amount of regular tripeptide. Subsequent investigation revealed that the cyclic product was slowly hydrolyzed. This observation is in accordance with previous findings.[23,14] Peptidomimetic **7** was prepared by a cyclization of resin bound phenylalanine using butyraldehyde followed by two subsequent condensation steps with Ala and Tyr protected amino acids. The product was obtained in a high purity and in 97% yield.[24]

The presented method enables incorporation of the imidazolidinone motif into a regular peptide sequence using both parallel and combinatorial solid-phase methodology. A new class of molecules derived from peptides is thus accessible. The heterocyclic moiety itself may yield useful biological properties through the conformational constraint of the peptide molecule that mimics specific steric features, while at the same time removing a hydrolyzable amide bond.

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18. General Boc solid-phase method used for preparation of a starting material **1** on *p*-methylbenzhydrylamine (MBHA) resin (1.1 mmol/g): neutralization with 5% DIEA in DCM (10 ml/100 mg of resin, 2×2 min), wash with DCM (5×10 ml/100 mg of resin); condensation using 6 equiv. of an amino acid derivative, 6 equiv. of HOBt and 6 equiv. of DICI in DMF (6 ml/100 mg of resin), deprotection with 55% TFA in DCM (10 ml/100 mg of resin, 20 min), wash with DCM (6×10 ml/100 mg of resin); L-amino acid derivatives used: Boc-Ala, Boc-Glu (γ-benzyl ester), Boc-Phe, Boc-Gly, Boc-Lys(*N*-ε-2-chlorobenzyloxycarbonyl), Boc-Leu, Boc-Pro, Boc-Thr(*O*-benzyl), Boc-Val, Boc-Tyr(*O*-2-bromobenzyloxycarbonyl).
19. Typical procedure for solid-phase formation of *N*-[1-(benzotriazole-1-yl)alkyl] intermediate **2**: Deprotected dipeptide-resin (100 mg) **1** was heated at 85°C in 12 ml of DMF with 10 equiv. of BtH and 10 equiv. of an aldehyde (formaldehyde, butyraldehyde) for 16 h, following decantation the procedure was repeated with fresh reagents.
20. The washed (DMF 3x, DCM 3x, MeOH 3x, each wash 10 ml) and thoroughly dried resin **2** was shaken with dry DCM (10 ml/100 mg of resin), 4 Å sieves and 3 equiv. of $BF_3{\cdot}Et_2O$ at rt overnight. Washing was carried out with DCM and MeOH (each 6×10 ml/100 mg of resin).
21. Compound **5a**: ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.99–170.69, 169.74, 167.32, 167.78, 156.75, 136.85–136.60, 130.53–130.45, 128.87–128.74, 128.32–128.22, 126.60–126.53, 124.29–124.04, 115.50–115.41, 58.61–58.29, 53.85–53.70, 51.72–51.34, 36.23, 34.57–34.46, 17.58, 15.63; compound **5b**: ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.85–169.79, 169.19, 167.95, 166.91, 156.82–156.76, 137.82, 137.26, 130.61, 130.21, 128.99–128.83, 128.32, 126.54, 124.21, 123.82, 115.63, 115.25, 72.27–72.12, 57.22–56.91, 54.02, 51.43, 36.63, 35.98, 35.16–34.99, 33.58, 18.80, 16.10, 13.87–13.68.
22. Compound **6**: MS m/z 452.2, found 453.2; ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.82–172.56, 171.28, 168.64, 167.74, 156.57–165.35, 137.84–137.73, 130.50, 130.30–130.17, 129.19–129.02, 128.10–128.02, 126.23, 124.76, 115.36–115.23, 54.35, 53.65–53.53, 50.39, 48.38, 37.62, 37.30, 36.25, 18.42, 13.53–13.36.
23. In our previous work (Ref. 15a) on *N*-1 substituted 4-imidazolidinone heterocycles, no Lewis acid was needed to complete cyclization. Also, we did not observe any significant hydrolysis of products. Herein, the reaction occurs on a primary amine, no other substituent is present at this stage of the synthesis, and we have made two observations: (i) low yields were obtained while not using any Lewis acid, and side products were identified indicating incomplete cyclization in this step; (ii) cleaved and analyzed samples of resins contained two main products, a cyclized intermediate, as well as a hydrolyzed dipeptidic byproduct. Closer observation showed that the stability towards hydrolysis of an alkylidene grouping joined to a secondary amine of compounds **3** is relatively low. Also, it has been reported that 4-imidazolidinones without an alkyl or acyl substituent on the nitrogen in the position 1 readily undergo hydrolysis (Ref. 14b). On the other hand, acylation on *N*-1 and formation of the second amide bond helps to stabilize the ring. For these reasons, we decided to pass over the characterization of these intermediates and continue with the next acylation step.
24. Compound **7**: MS m/z 452.2, found 453.5; ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.29, 170.79–170.29, 167.74, 156.72–156.62, 136.80–136.19, 130.80–130.40, 130.16–130.11, 128.51, 128.24–128.05, 127.01, 126.78–126.55, 124.60–124.22, 115.44–115.28, 68.50, 59.66–59.57, 53.46–53.17, 45.68–45.57, 38.08, 36.37, 17.98–17.69, 17.11–16.94, 13.66–13.49.