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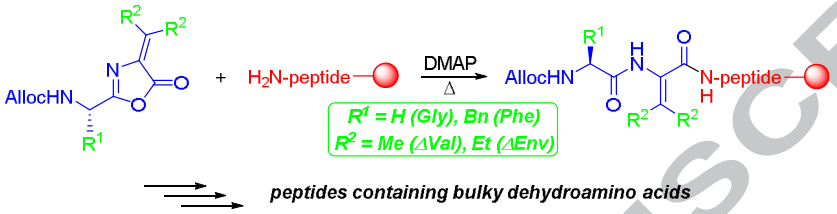

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Solid-phase synthesis of peptides containing bulky dehydroamino acids

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ABSTRACT

A method of incorporating bulky α,β-dehydroamino acids such as dehydrovaline and dehydroethylnorvaline into peptides via solid-phase peptide synthesis is reported. The key step involves ring opening of an azlactone (i.e., oxazolone) containing the dehydroamino acid by the amino group of a resin-bound peptide. The use of Alloc-protected azlactones was key to the success of the process.

α,β-Dehydroamino acids (ΔAAs) are characterized by planar geometry and restricted rotational freedom relative to standard amino acids. They can enhance the proteolytic stability of peptides that contain them, presumably by altering the shape of the backbone and by stabilizing folded states through their rigidifying effect.[1] The conformational preferences of ΔAAs that contain at least one hydrogen atom at the β carbon (e.g., ΔAla, ΔAbu, ΔPhe) have been well-studied, and design rules governing their inclusion in secondary structures have been established.[2,3] However, much less is known about bulky ΔAAs that are fully substituted at the β carbon (e.g., ΔVal), which should exhibit a more pronounced rigidifying effect on peptides due to their high levels of $A_{1,3}$ strain (Figure 1).[4] While the structures of simple ΔVal derivatives[5] and ΔVal-containing di-, tri-, and tetrapeptides[6] have been examined, we are unaware of any studies of larger peptides that include this residue. This gap in knowledge can be attributed to a lack of methods for incorporating bulky ΔAAs into peptides via solid-phase synthesis techniques.

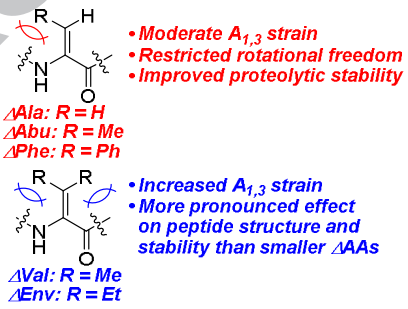

Figure 1. Normal and bulky dehydroamino acids.

In the course of our efforts to synthesize natural products that contain tetrasubstituted ΔAAs,[7] we became interested in examining the effects of bulky residues such as ΔVal and its homologue dehydroethylnorvaline (ΔEnv, Figure 1) on the structures of peptides. As a first step in this direction, we have developed protocols for the efficient solid-phase synthesis of simple peptides that include these bulky ΔAAs. This work sets the stage for the preparation and study of more complex ΔAA-containing peptides including therapeutically relevant compounds.

Our synthetic strategy is summarized in Scheme 1 and was inspired by the fact that *C*-terminal dehydroamino acids are rapidly transformed into azlactones (i.e., oxazolones) upon activation of their carboxylate group.[8] Although this process causes the stereochemical scrambling of unsymmetrical ΔAAs such as *E*- or *Z*-ΔIle,[9] it can be used to facilitate couplings to symmetrical ΔAAs such as ΔVal and ΔEnv. Thus, we envisioned that dipeptides **1** bearing either ΔVal or ΔEnv at the C terminus would undergo dehydration and cyclization in a single pot to generate azlactones **2**. Then, heating **2** in the presence of a resin-bound peptide should trigger a coupling reaction, furnishing elongated resin-bound peptides **3**. Next, subjection of **3** to standard solid-phase peptide synthesis (SPPS) protocols followed by cleavage from the resin and purification would provide the targeted peptides with bulky ΔAAs located at the desired positions. Prior reports of thermally promoted ring openings of azlactones by amines in solution[10] provided some support for our strategy. However, the lack of examples involving resin-bound amines was concerning.

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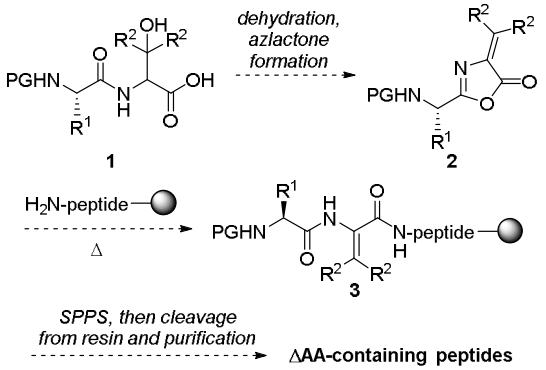
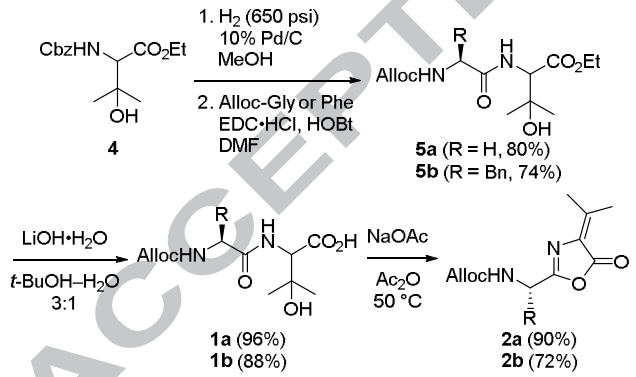


Scheme 1. Strategy for synthesis of ΔAA-containing peptides via SPPS.

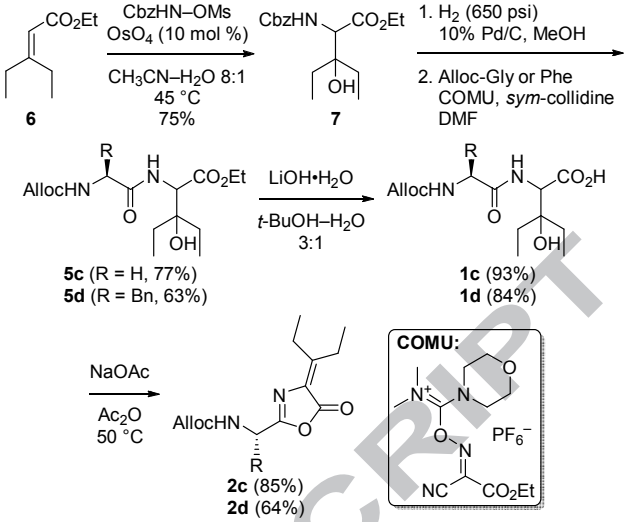
Our initial attempts to execute this plan employed Fmoc-protected dipeptides of type **1**. Although formation of the azlactones was facile, heating solutions of these species in the presence of resin-bound peptides yielded only trace amounts of the coupled products according to MS analysis. Further investigation suggested that the Fmoc group was not stable under the reaction conditions. Accordingly, we decided to examine Alloc-protected substrates, since this group would presumably be more stable than the Fmoc group under the weakly basic coupling conditions. Moreover, cleavage of the Alloc moiety from solid-supported peptides has been accomplished under conditions that are compatible with the acid-labile side-chain protecting groups that are typically employed in Fmoc-based SPPS.[11]

Based on this logic, we constructed ΔVal-containing dipeptides **1** and azlactones **2** as outlined in Scheme 2. Hydrogenolysis of racemic Cbz-protected β-OHVal derivative **4**[7a] and coupling of the resulting amine with Alloc-Gly or Alloc-Phe afforded dipeptides **5a** and **5b**, respectively, in good yields. Saponification furnished the free acids **1a** and **1b**, which underwent facile dehydration and cyclization upon exposure to Ac_2O and NaOAc.[12] The azlactones **2** were typically used in subsequent reactions without purification due to their sensitivity to SiO_2.



Scheme 2. Synthesis of ΔVal-based azlactones.

In preparation for synthesizing the corresponding ΔEnv-containing azlactones, we prepared racemic β-hydroxy ethylnorvaline derivative **7** in one-step via base-free aminohydroxylation[7,13] of known enoate **6**[14] (Scheme 3). This compound was transformed into azlactones **2c** and **2d** via the same sequence employed to convert **4** into **2a** and **2b**. COMU[15] was found to be superior to EDC•HCl and HOBt for mediating peptide couplings of the hindered amine derived from **7**. The slightly lower yields of the couplings and dehydration–cyclization reactions in the ΔEnv series are presumably a consequence of the bulkier nature of this amino acid relative to ΔVal.



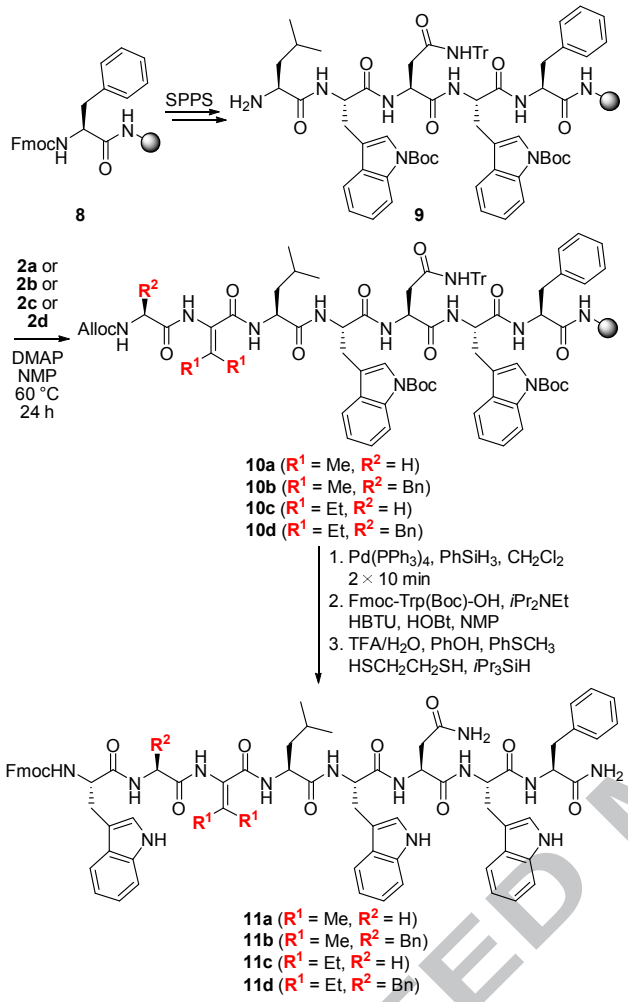
Scheme 3. Synthesis of ΔEnv-based azlactones.

With the requisite azlactones **2a–d** in hand, we explored their incorporation into resin-bound peptides. We wished to use a therapeutically relevant peptide as a system for determining the viability of our synthetic strategy. Accordingly, we decided to synthesize analogues of the *C*-terminal region of enfuvirtide, an FDA-approved inhibitor of HIV membrane fusion.[16] Thus, attachment of Fmoc-Phe-OH to Rink amide resin and elaboration using standard Fmoc-based SPPS techniques afforded resin-bound pentapeptide **9** (Scheme 4). Different solvents (DMF versus NMP), reaction temperatures (50 °C versus 60 °C), and additives (DMAP versus no additive) were then evaluated in the coupling of **9** with azlactone **2a**. Analysis of the reactions by LC/MS revealed that heating the mixture at 60 °C in NMP for 24 h in the presence of DMAP yielded the best results, although the coupling also proceeded in the absence of the additive. Therefore, these conditions were employed to mediate the ring openings of azlactones **2b–2d** by **9**. Each reaction afforded the desired product as evidenced by MS, but the yields decreased somewhat as the size of the azlactones increased (vide infra).

The Alloc groups of peptides **10a–d** were then removed using conditions ($Pd(PPh_3)_4$, $PhSiH_3$) that were previously developed for the deprotection of resin-bound peptides.[11] This process was repeated to ensure complete conversion. Coupling of the resulting resin-bound amines to Fmoc-Trp(Boc)-OH was then accomplished under standard conditions. Finally, TFA-mediated deprotection and cleavage of the octapeptides from the solid support furnished the targeted compounds **11a–11d**, which were purified via reverse-phase HPLC.

The nature of SPPS renders it impossible to precisely determine the yield of a single step in a synthetic sequence. Notwithstanding, we were interested in comparing the yields of the couplings of azlactones **2a–2d** with **9**. The HPLC traces of the crude final products **11a–11d** provided us with a qualitative means of making this comparison. The trace for crude **11a**, which was derived from the smallest azlactone **2a**, was very clean with only a handful of minor impurities present.[17] The traces for crude **11b** and **11c**, which are derived from medium-sized azlactones **2b** and **2c**, were similar to each other and showed a few more impurities than the trace for **11a**. Still more impurities were visible in the trace for crude **11d**, which is obtained from the largest azlactone **2d**. Although it is possible that the Alloc deprotection, Trp coupling, and final cleavage/deprotection steps could have proceeded with varying yields, it is likely that the differences in the amounts of **11a–11d** are primarily due to the ring opening reaction proceeding more smoothly with less-

hindered azlactones. Nonetheless, it is important to note that all four targeted octapeptides were abundant and were easily purified to homogeneity.



Scheme 4. Solid-phase synthesis of ΔAA-containing peptides.

In summary, we have devised a protocol for incorporating bulky tetrasubstituted α,β-dehydroamino acids into peptides via solid-phase peptide synthesis. Our method capitalizes on the facile generation of azlactones (i.e., oxazolones) from dipeptides that possess *C*-terminal β-hydroxy amino acids via a one-pot dehydration–cyclization process. The azlactone rings are readily opened by the amino groups of resin-bound peptides. Efforts to improve the couplings of hindered azlactones and to synthesize full-length therapeutic peptides by this method are in progress and will be reported in due course.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.xxxx.xx.xxx. These data include experimental procedures, spectral data, and HPLC traces.

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17. The HPLC traces can be found in the Supplementary Data section.