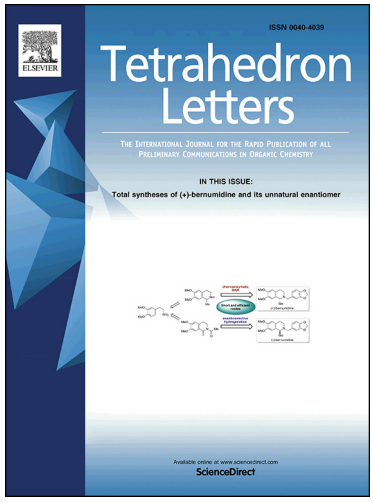

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A mild capping method for SPPS on the *N*-methyl diaminobenzoyl linker: synthesis of an *N*-acyl urea appended *C. elegans* neuropeptide

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ABSTRACT

In this manuscript, we establish the susceptibility of the *N*-methyldiaminobenzoyl linker to undergo undesired acylation during standard peptide capping with acetic anhydride. Successive capping treatments led to problematic levels of linker incapacitation. We describe a mild, inexpensive alternative capping strategy that is completely selective for the N terminus with no acylation of the linker detected for any of the substrates evaluated. The utility of this protocol is demonstrated via the synthesis of the CAPA-PVK-1 consensus sequence of the *C. elegans* neuropeptide-like protein precursor peptide NLP-44.

Introduction

As methods for the chemical synthesis of complex peptides and proteins continue to evolve, these approaches are becoming increasingly viable for large scale production of homogenous peptide and protein-based pharmaceutical agents.[1] Toward this end, diaminobenzoyl (Dbz) linkers have gained traction in the synthetic community as versatile precursors for the synthesis of peptide thioesters[2] for native chemical ligation (NCL),[2,3] C-terminally modified peptides,[4] and macrocyclic peptides[5] via solid phase peptide synthesis (SPPS). Incomplete coupling reactions can lead to the formation of problematic deletion products that are difficult to separate from the desired peptide. Capping after an incomplete coupling via treatment with acetic anhydride and Hünig's base[6] prevents further elongation of these sequences (i.e., **1** → **2**, **Scheme 1A**) during Boc-[7] or Fmoc-SPPS.[8] The resulting peptides are both shorter than the target peptide and have a different charged state, changing their retention time and facilitating the purification of the desired target (**5**). We hypothesized that the unactivated *N*-methyl diaminobenzoyl (MeDbz) linker would be susceptible to acylation under these conditions (i.e., **6** → **7**, **Scheme 1B**), resulting in low yields of the desired peptide from the resin. In this manuscript, we establish the cumulative negative effect of performing capping reactions in presence of the MeDbz linker and present a mild, inexpensive protocol for the selective capping of the N terminus using benzoic acid, *N*,*N*'-dicyclohexylcarbodiimide (DCC), and Hünig's base. We demonstrate the limitations of acetic anhydride capping and the effectiveness of benzoic acid capping in the context of the synthesis of a putative neuropeptide derived from the *C. elegans* neuropeptide-like protein precursor NLP-44.

The diaminobenzoyl (Dbz) linker has become widely used in solid-phase peptide synthesis. However, a limitation of this linker is the formation of branched side-products (i.e., peptide growth on both aryl amines) during SPPS.[2a-c] Protection of one nitrogen atom by an Alloc group avoids branching and allows for capping during peptide synthesis.[2b] However, Alloc-Dbz can decompose to the prematurely activated N-acyl urea (Nbz), leading to peptide loss via formation of C-terminal piperidinyl amide side products throughout the synthesis.[9] Alternatively, capping can be performed in presence of a nitrobenzoyl analog, but a reduction step must be performed prior to activation of this linker.[2c] In 2015, a second-generation Dbz linker, *N*-methyldiaminobenzoyl (MeDbz, **6**) was reported (**6**, **Scheme 1B**).[2d] The presence of an *N*-methyl group supresses the formation of branched products because of the increased steric hindrance of the secondary amine relative to the original primary amine. However, we hypothesized that with more reactive reagents, such as anhydrides, the secondary amine would be prone to acylation (i.e.; AcMeDbz, **7**). The AcMeDbz moiety cannot be activated toward nucleophilic displacement, and so the target peptide (**8**) cannot be accessed following inadvertent linker acylation. We envisioned that employing milder carboxylic acid activation conditions would allow selective functionalization of the N-terminal amine following incomplete coupling reactions without also acylating the linker.

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