



Expedient on-resin synthesis of peptidic benzimidazoles

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ABSTRACT

The benzimidazole moiety is a ubiquitous pharmacophore present in numerous anthelmintic, antibacterial, antiviral, antineoplastic, and antifungal drugs. While the polypharmacology of this heterocycle has spurred the development of numerous solution-phase syntheses, only a handful of disparate and inefficient methods detailing its synthesis on-resin have been reported. Here we report the concise and expedient syntheses of internal and C-terminal peptidic benzimidazoles – an emerging class of peptide deformylase (PDF)-inhibiting antimicrobials. This method benefits from being performed wholly on solid-phase at room temperature resulting in minimal purification and tolerance of temperature-sensitive functionality.

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The benzimidazole moiety has proven an invaluable pharmacophore in the development of novel bioactive compounds for the treatment of a diverse range of diseases.^{1,2} The chemical pluripotency of this structural motif has led to its incorporation into a number of antineoplastic,^{3–13} antibacterial,¹⁴ antifungal,¹⁴ anthelmintic,^{15–19} and antiviral agents,^{20,21} in addition to being an integral part of some successful antihypertensives²² and anti-inflammatories.²³ In the context of antimicrobial therapies, efficacious benzimidazole-containing molecules halt ribosomal protein synthesis in bacteria, protozoans, and some fungi by inhibiting peptide deformylase (PDF) – an essential metalloprotein required for N-terminal deformylation of pro-proteins.^{24,25} This ubiquitous and heavily conserved target, while present in higher eukaryotes,²⁶ is wholly unnecessary for protein synthesis in humans²⁷ and therefore represents a valuable target in the hunt for novel antimicrobials with large therapeutic indices.^{24,25,28} Among a variety of heterocyclic cores displaying PDF-inhibition,²⁹ the benzimidazolyl structure generalized in Scheme 1 was shown to be particularly efficacious.^{30,31}

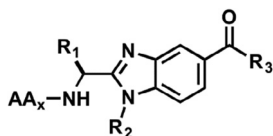
Inspired by the far-ranging medicinal utility of benzimidazoles and in light of our recent work using benzimidazole-like C-terminal functionality (colloquially “Dbz”), as well as second generation “R-Dbz” functionalities, as thioester precursors for use in native

chemical ligation (NCL),^{32–34} we endeavored to streamline the synthesis of peptidic benzimidazoles by using “R-Dbz” linkers or “R-Dbz”-loaded resin and standard solid-phase peptide synthesis (SPPS) resin-cleavage conditions of trifluoroacetic acid (TFA) and scavengers, Scheme 2.

Generally, the synthesis of amino acids containing benzimidazole functionality proceeds via solution-phase reduction of a 3-nitro-4-aminophenylalanine³⁵ or similar di-nitro compound³⁶ with subsequent heating in acetic acid to induce dehydration. This strategy has been adapted for solid phase peptide synthesis using Fmoc-protected 3-nitro-4-aminophenylalanine and on resin reduction with tin(II) chloride, followed by condensation of the diamine onto a range of aldehydes.³⁷ In addition, C-terminal benzimidazoles have previously been accessed by solution-phase coupling of a peptide acid and 1,2-diaminobenzene followed by dehydration in hot acetic acid.^{38,39} Overall, these methods rely on solution phase amide couplings and high temperatures in acidic media to generate benzimidazoles solely on the C-terminus of peptides. These methods necessitate multiple purifications, thereby lowering overall yield, in addition to requiring thermally-insensitive pendant functionality. Herein, we present a method to circumvent these issues by using high-yielding, all-solid-phase couplings starting from “R-Dbz” linkers to generate the benzimidazole moiety at both the C-terminus and along the peptide backbone with the requirement of simple resin cleavage and a single purification. These linkers have the significant advantage of not requiring protection during peptide synthesis, thereby removing a deprotection

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Scheme 1. General structure of a number of benzimidazole-containing peptide antibacterials targeting peptide deformylase (PDF).

step that was required for previous on-resin approaches to benzimidazole synthesis.^{34,37}

The dependence of the dehydrative ring closure on the amino acid side chain pendant to the nascent benzimidazole was investigated using the test peptide H-KYEAX-MeDbz-NH₂, Fig. 1. Following SPPS, simply leaving the peptide mixture under standard Fmoc-SPPS cleavage conditions (TFA/scavengers, Fig. 1) for 24 h instead of the normal 1–2 h afforded cyclized Dbz (the benzimidazole) in generally good conversions. After 24 h, crude peptide was obtained by the addition of cold diethyl ether, centrifugation, and decanting of the organic phase. The resulting pelleted peptide was then analyzed using liquid chromatography – mass spectrometry (LC-MS) (see [Supporting Information](#)). While conversion of a single species cannot easily be determined by HPLC integration at 214 nm due to differing absorption properties of the two products, but the error caused by this difference should be relatively uniform across all 20 peptides. Therefore, the ratio of the area under the benzimidazole peak to the summed area of the benzimidazole and Dbz peaks was used as a metric for comparing relative conversion between the 20C-terminal amino acid variations, Fig. 1. Those amino acids containing sterically bulky side chains (Ile, Val, Trp, Phe, and Tyr) or are backbone constrained, Pro, showed low cyclization efficiency. However, when heating is compatible with other present functionalities, difficult cyclizations can be forced by heating in acetic acid (see [Supporting Information Page S-27](#)).

The cyclization efficiency of Dbz-derivatives containing electronically differentiated aniline substitution was investigated using a different test peptide (Ac-LYRAG-Dbz_{R2}-NH₂), Fig. 2. Comparing the conversion of Dbz derivatives where R = Me vs. Ph over 1 h revealed a clear rate-enhancement using the relatively electron poor, Ph-substituted Dbz. Employing electron-rich 2,3,4-trimethoxyphenyl-Dbz showed conversions similar to Me-Dbz. Taken together, these results showcase how electronic derivation at this position may be exploited to coax reluctant Dbz species into dehydrating.

The utility of this method for the creation of drug-like peptides ([Scheme 1](#)) was explored by the synthesis of peptides containing the benzimidazole moiety along the amide backbone. Peptides H-AG-MeDbz-DA-NH₂ and H-AG-PhDbz-DA-NH₂ were synthesized via standard SPPS and, upon standing in cleavage cocktail for 24 h at room temperature, converted to the benzimidazole, Fig. 3. Mirroring the previous result with C-terminal PhDbz peptide (Fig. 2) the internal PhDbz-containing peptide converted cleanly to benzimidazole, while the MeDbz-containing peptide retained traces of starting material.

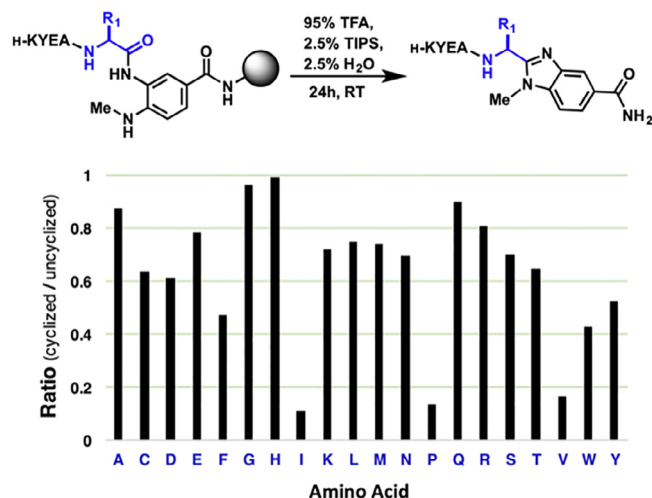


Figure 1. Relative extent of benzimidazole cyclization as a function of pendant amino acid side chain. Relative conversion ratio derived from integration of 214 nm peaks; [benzimidazole]/[benzimidazole + Dbz].

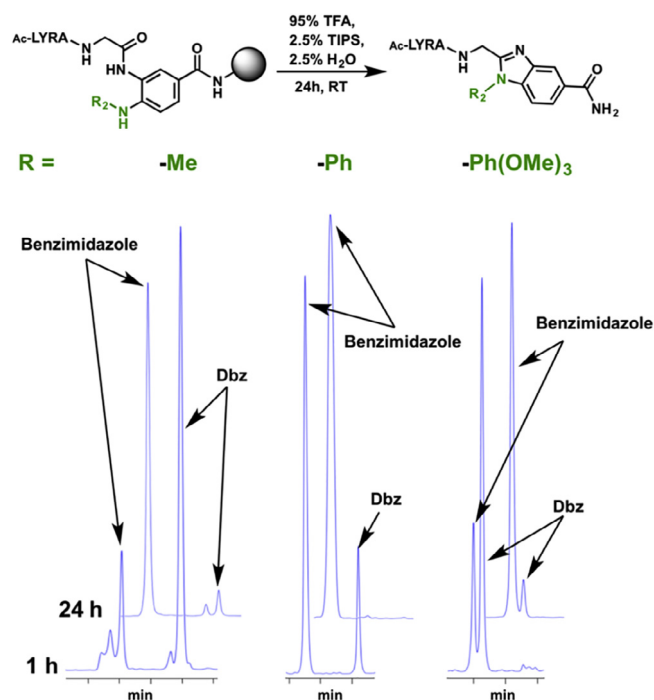
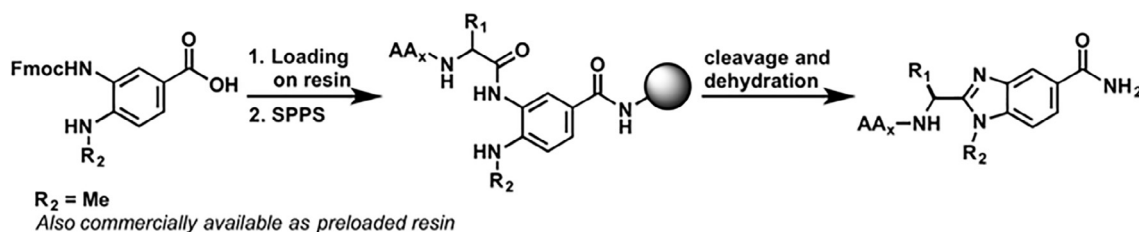


Figure 2. Crude HPLC traces of cyclization of Dbz derivatives containing varied aniline-substitution to benzimidazoles after 1 h or 24 h in a standard SPPS cleavage cocktail.



Scheme 2. Streamlined strategy for the synthesis of peptidic benzimidazoles.

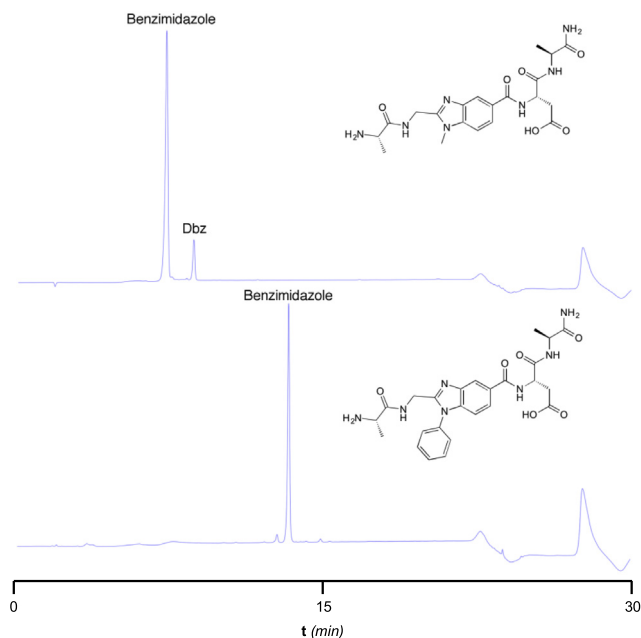


Figure 3. Crude HPLC traces of the syntheses of peptides containing internal MeDbz and PhDbz moieties via SPPS and their conversion to backbone-benzimidazole peptides upon cleavage from resin under standard conditions.

An operationally simple method to generate C-terminal and internal benzimidazolyl peptides using standard SPPS conditions has been developed. Linear assembly of “R-Dbz” peptides by Fmoc SPPS introduces the obligate aminoanilide without need for additional synthetic manipulations. Subsequent resin cleavage and side chain deprotection by TFA yields peptide benzimidazole products cleanly at room temperature. As conversion to benzimidazole occurs readily during TFA cleavage conditions, no additional handling steps are added compared to a standard Fmoc-SPPS procedure. This approach presents a significant level of convenience over previous methods and should be compatible with heat and redox sensitive functional groups. This method has the potential to facilitate the synthesis, incorporation, and evaluation of the benzimidazole pharmacophore in novel antineoplastic and antimicrobial agents.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bmcl.2018.04.062>.

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