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Orthogonally Protected Thiazole and Isoxazole Diamino Acids: An Efficient Synthetic Route

Jeffrey D. Butler, Keith C. Coffman, Kristin T. Ziebart, Michael D. Toney, and Mark J. Kurth*[a]

Heterocyclic and heteroaromatic amino acids (HAAs) are central to the motifs of peptide antibiotics, including microcin B17, nostocyclamide, telomestatin, and thiostrepton.[1] α-Amino acids undergo cyclization and oxidation to form heteroaromatic rings, notably, thiazoles, oxazoles, indoles, and pyridines, which give rise to well-documented antibiotic activity.[1] Few of these targets have succumbed to total synthesis due, in large part, to the demand for orthogonally protected HAA building blocks.[1b] In contrast, commercial orthogonally protected natural amino acids, most commonly lysine and aspartic acid, are routinely used as the branch point in the synthesis of branched or cyclic peptide and oligosaccharide mimetics[2–6] (Figure 1a). Similarly, these agents see action in the ligation of imaging agents (Figure 1b) and in diversity-oriented syntheses (e.g., **I**→**II**, Figure 1c).[7,8] However, the stringent orthogonal chemistry requirements, especially in solid-phase synthesis, make optimization at this branch-point region challenging.

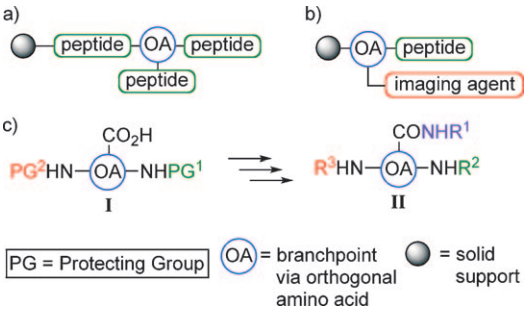

Figure 1. Common motifs and methods employing an orthogonally protected diamino acid, which include a) branched peptides, b) ligated imaging agents, and c) diversity-oriented methods.

Surprisingly, methods to generate new heterocyclic non-natural amino acids with an additional orthogonally protected amino group (e.g., diamino acids), are still rare.[6,9d] Non-natural conformationally restrictive amino acids have potential in the discovery of new peptidomimetics and in efforts to improve the pharmacological and protease resistant properties of bioactive peptides.[1b,9] There is demand for practical HAA syntheses that deliver orthogonally protected diamino acids compatible with the traditional solid and solution phase 9-fluorenylmethoxycarbonyl (Fmoc) protection strategy. Thus our focus herein is on the development of short, high yielding syntheses delivering heteroaromatic mono- and diamino acids from readily available starting materials.

Herein, we report an efficient synthesis yielding thiazole- and isoxazole-based HAAs from β-amino acids. This strategy allows for orthogonal carbamate protection that permits independent synthetic manipulation (Figure 1). Further, the viability of the synthesized HAAs as branch-point amino acids is demonstrated in the solid-phase synthesis of an inhibitor of two chorismate utilizing enzymes, anthranilate synthase (AS) and isochorismate synthase (IS). This inhibitor shows two- and threefold better activity than its lysine predecessor in the inhibition of AS and IS, respectively.

A wide variety of β-amino acids are commercially available and considerable synthetic effort has been focused on producing novel optically active β-amino acids.[10] This availability makes β-amino acids an attractive starting material for this work. As outlined in Figure 2, our synthetic method began by carbamate protection (Teoc, Boc, Cbz, and Alloc) of β-alanine following literature procedures.[11] These protected acids were subjected to coupling conditions to install the Meldrum acid moiety in 94–98% yield. Intramolecular cyclization of **1a–d**→**2a–d** is accomplished quantitatively in EtOAc at reflux via a presumed ketene intermediate.[12] In a modification of Suzuki's general method of cyclocondensa-

[a] Dr. J. D. Butler, K. C. Coffman, Dr. K. T. Ziebart, Prof. M. D. Toney, Prof. M. J. Kurth
Department of Chemistry, University of California, Davis
One Shields Avenue, Davis, CA 95616 (USA)
Fax: (+1) 530-752-8995
E-mail: mjkurth@ucdavis.edu
Homepage: http://chemgroups.ucdavis.edu/~kurth/

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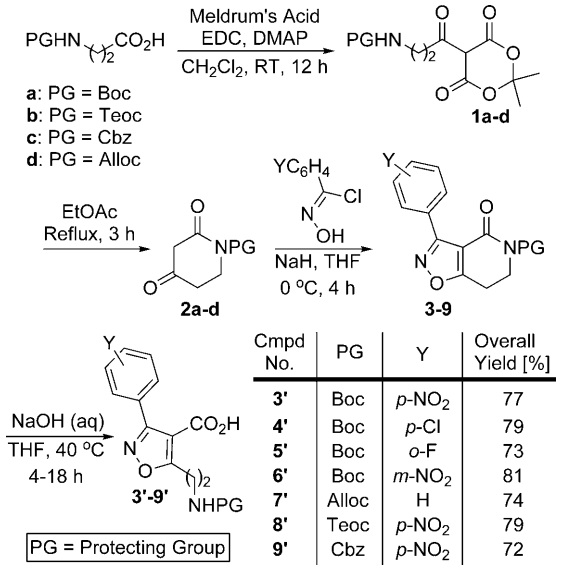
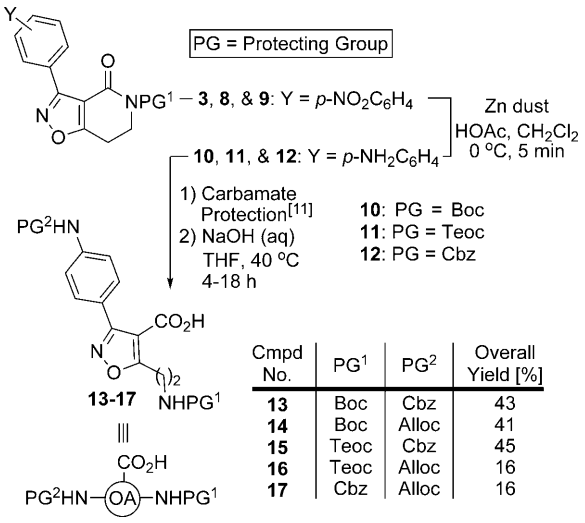


Cmpd No.	PG	Y	Overall Yield [%]
3'	Boc	*p*-NO_2	77
4'	Boc	*p*-Cl	79
5'	Boc	*o*-F	73
6'	Boc	*m*-NO_2	81
7'	Alloc	H	74
8'	Teoc	*p*-NO_2	79
9'	Cbz	*p*-NO_2	72

Figure 2. General synthetic method of isoxazole-based amino acids. Boc = *tert*-butyloxycarbonyl, Teoc = 2-(trimethylsilyl)ethoxycarbonyl, Cbz = carbobenzyloxy, Alloc = allyloxycarbonyl.

tion,[13] compounds **2a–d** were treated with strong base (NaH) and α-chlorobenzaldo oximes to generate isoxazole adducts **3–9** in 83–90% yield. These mixed imides (**3–9**) were efficiently hydrolyzed to yield amino acids **3′–9′** in 72–81% overall yield without carbamate deprotection.

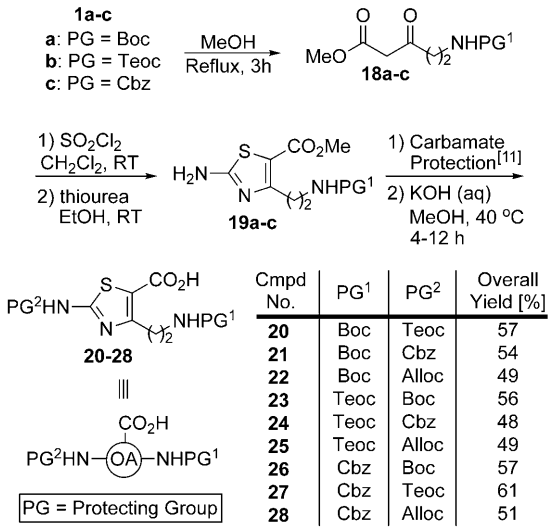
Our approach to diaminoisoxazole and thiazole HAAs began similarly to the monoamino acids of Figure 2. As delineated in Figure 3, zinc reduction of intermediates **3**, **8**, and **9** (when Y = *p*-NO_2) delivered aniline analogues **10–12**. These anilines were carbamate protected with Alloc and Cbz; subsequent imide hydrolysis delivered a collection of orthogonally protected bis-carbamate isoxazole-based HAAs (**13–17**) in 16–45% overall yield as tabulated in Figure 3.



Cmpd No.	PG^1	PG^2	Overall Yield [%]
13	Boc	Cbz	43
14	Boc	Alloc	41
15	Teoc	Cbz	45
16	Teoc	Alloc	16
17	Cbz	Alloc	16

Figure 3. General synthetic method of isoxazole-based orthogonally protected diamino acids.

To deliver orthogonally protected thiazole HAAs, intermediates **1a–c** (Figure 4) were heated in methanol at reflux to quantitatively deliver methyl esters **18a–c**.[12b] These β-keto esters were α-chlorinated by using sulfuryl chloride and, without purification, condensed with thiourea to give thiazoles (**19a–c**) in two-step yields of 75–79%.[14] These 2-amino thiazoles were carbamate protected to give orthogonally protected esters that were subsequently saponified to deliver a collection of thiazole-based orthogonally protected diamino acids (**20–28**) as summarized in Figure 4 in an efficient 48–61% overall yield. In addition, these methods have been successful in preparing >5 g of **25** in comparable overall yield.

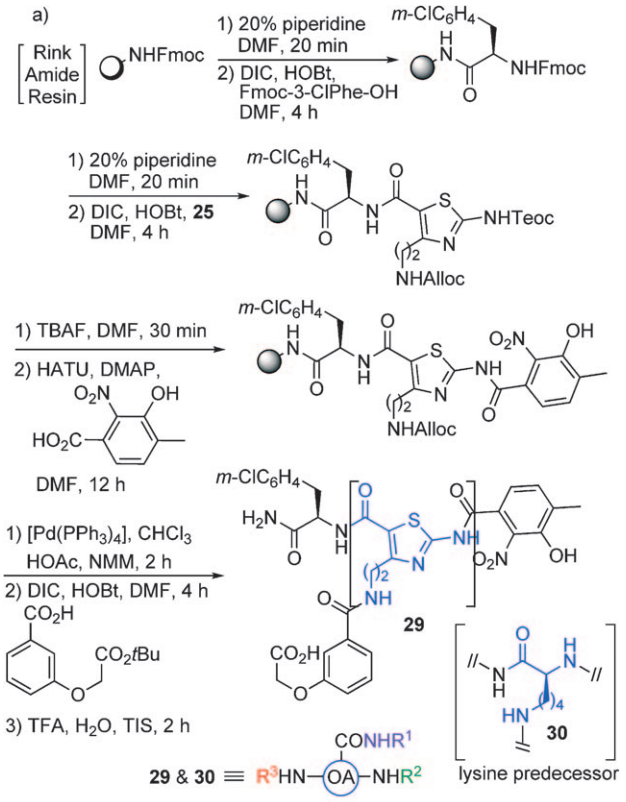


Cmpd No.	PG^1	PG^2	Overall Yield [%]
20	Boc	Teoc	57
21	Boc	Cbz	54
22	Boc	Alloc	49
23	Teoc	Boc	56
24	Teoc	Cbz	48
25	Teoc	Alloc	49
26	Cbz	Boc	57
27	Cbz	Teoc	61
28	Cbz	Alloc	51

Figure 4. General synthetic method of thiazole-based orthogonally protected diamino acids.

To explore how efficiently our diamino HAAs perform in a typical branched synthesis, a solid-phase synthesis of a staged inhibitor analogue (**29**) was undertaken, as depicted in Figure 5. These inhibitors are of particular interest to our group[8] and are an ideal test case for the employment of these novel orthogonal diamino acids.

To synthesize **29**, Rink amide resin was Fmoc deprotected and *N*-Fmoc-3-chloro-L-phenylalanine was coupled by using standard solid-phase peptide chemistry conditions. After subsequent Fmoc deprotection, the Teoc/Alloc orthogonal HAA **25** was coupled by using DIC and HOBt. Treatment with TBAF in DMF removed the Teoc protecting group[11] revealing a free amine that was subsequently coupled using dehydrative conditions with 3-hydroxy-4-methyl-2-nitrobenzoic acid. The Alloc protecting group was removed under Pd^0 conditions.[11] Finally, DIC- and HOBt-mediated coupling of 3-[(*tert*-butoxycarbonyl)methoxy]benzoic acid completed the independent functionalization of both amines. Resin cleavage and HPLC purification gave **29** in 91% purity and 39% overall yield, which confirms that these or-



b)

Cmpd No.	AS K_i [μM]	AS K_i' [μM]	IS K_i [μM]
29	9.5 ± 1.6	34 ± 10	73 ± 7
30	20 ± 2	54 ± 4	210 ± 29

Figure 5. a) Synthetic method of **29** b) IS and AS inhibition data for compounds **29** and **30**. DIC = diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, TBAF = tetrabutylammonium fluoride, HATU = *O*-(7-azabenzotriazol-1-yl)tetramethyluronium hexafluorophosphate, DMAP = 4-dimethylaminopyridine, NMM = *N*-methylmorpholine, TFA = trifluoroacetic acid, TIS = triisopropylsilane.

thogonal diamino HAAs are compatible with standard Fmoc peptide chemistry conditions.

AS and IS are structurally homologous chorismate-utilizing enzymes, and they are excellent antimicrobial drug targets due to their absence in humans and their roles in bacterial and apicomplexan parasite cell survival and/or virulence.[15] Compound **29** is a structural analogue of a previously discovered inhibitor with a lysine scaffold (**30**).[8] The inhibition properties of **29** are similar to **30**, but, satisfyingly, the exchange of α-lysine for thiazole HAA gives a significant two- and threefold increase in potency against AS and IS, respectively (Figure 5b). This result represents a step forward in our program to find inhibitors of chorismate-utilizing enzymes. More importantly, this application underscores the value of these HAAs in the development and optimization of tight-binding biological ligands in other discovery efforts.

The goal of developing high-yielding syntheses of heteroaromatic mono- and diamino acids from readily available starting materials has been realized. Our strategy delivers orthogonally carbamate protected diamino HAAs that are of value for diversity-oriented methods and allow for branched peptide/imaging agent synthesis. Their viability as branch-point amino acids has been successfully demonstrated in the solid-phase synthesis of an inhibitor (**29**) of anthranilate synthase (AS) and isochorismate synthase (IS) with improved potency over its lysine predecessor.

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Keywords: amino acids · inhibitors · isoxazoles · protecting groups · thiazoles

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