Published in final edited form as:

Angew Chem Int Ed Engl. 2020 January 07; 59(2): 890–895. doi:10.1002/anie.201911886.

Co(III)-Catalyzed C—H Amidation of Dehydroalanine for the Site-Selective Structural Diversification of Thiostrepton

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Abstract

Thiostrepton is a potent antibiotic against a broad range of Gram-positive bacteria, but its medical applications have been limited by its poor aqueous solubility. In this work, the first C(sp²)—H amidation of dehydroalanine (Dha) residues was applied to the site selective modification of thiostrepton to prepare a variety of derivatives. Unlike all prior methods for the modification of thiostrepton, the alkene framework of the Dha residue is preserved and with complete selectivity for the Z-stereoisomer. Additionally, an aldehyde group was introduced by C—H amidation, enabling oxime ligation for the installation of an even greater range of functionality. The thiostrepton derivatives generally maintain antimicrobial activity, and importantly, eight of the derivatives displayed improved aqueous solubility (up to 28-fold), thereby addressing a key shortcoming of this antibiotic. The exceptional functional group compatibility and site selectivity of Co(III)-catalyzed C(sp²)—H Dha amidation suggests that this approach could be generalized to other natural products and biopolymers containing Dha residues.

Graphical Abstract

Co(III)-catalyzed C—H amidation was used for the site-selective modification of the antibiotic thiostrepton. A range of amide groups was installed with 1,4,2-dioxazolone coupling partners. The introduction of an aldehyde group by C—H amidation enabled modular incorporation of additional functionality by oxime formation. A number of the thiostrepton derivatives showed greatly improved aqueous solubility while maintaining antibacterial activity.

Keywords

C—H activation; cobalt; antibiotic; chemoselectivity; natural product

Introduction

Thiostrepton **1** (Figure 1, top) is a potent antibiotic synthesized by several strains of Gram-positive microbes.^{1,2} Although it is used as an active component in topical veterinary antibiotics, applications to human medicine are limited owing to poor physicochemical properties, particularly low aqueous solubility.^{1a} Although site-specific modification of **1** is a possible approach for improving its aqueous solubility, architectural complexity and a high density of delicate functionalities provide a formidable barrier to successful, site-selective chemical modification.^{3,4}

The dehydroalanine (Dha) residues characteristic of **1** and other thiopeptides are reactive sites, most notably as Michael acceptors for a variety of nucleophilic species.⁵ Previously, several studies have been carried out on additions to the Dha residues in **1** (Figure 1, middle).^{6,7} Variable Dha site selectivity was observed depending on the transformation and reaction conditions, with notable site and diastereoselectivity achieved for Rh-catalyzed arylboronic acid additions.^{6b} Importantly, many of the synthesized thiostrepton derivatives maintained potent antibacterial activity with only moderate reductions relative to **1**. However, hydrophobic functionality has generally been introduced, including in recent work involving cycloaddition to the Dha residues,^{7a} and aqueous solubility has not been reported for any of these derivatives of **1**. Moreover, physicochemical properties of derivatives of **1** have not been characterized, except for studies by Honek and coworkers on several thiol Michael adducts showing qualitative increases in overall polarity by RP-HPLC and improvements in theoretical logP.^{6d}

The previously investigated chemical modifications of the Dhas in **1** resulted in loss of the alkene framework to give modified residues with sp³ rather than sp² hybridization. We sought a complementary method that instead maintains the unsaturated character of the Dha residue through catalytic C(sp²)—H functionalization.⁸ This type of modification also avoids epimeric mixtures that can complicate isolation of pure stereoisomers for biological studies. We specifically pursued methods to install polar functionality that would be beneficial to aqueous solubility. Evaluation of a number of catalysts and coupling partners was required to achieve this goal.

Here we report the first application of C(sp²)—H amidation of Dhas for the site selective introduction of amide functionality at the terminal Dha residue of **1** (Dha1; Figure 1, bottom). In addition to defining a new threshold for functional group tolerance in Cp*Co(III)-catalyzed C—H functionalization reactions, a wide range of functionality could be incorporated by C—H amidation using dioxazolone coupling partners. Moreover, by introducing an aldehyde group with C—H amidation, oxime ligation could be employed to install an even greater range of functionality. Many of the analogs maintained potent antibacterial activity with only a moderate reduction relative to thiostrepton perhaps because Dha1 is located in a solvent exposed region of thiostrepton with modifications to this region

generally well-tolerated.^{6b-d,7a} Most importantly, many analogs showed significantly greater solubility in pH 7.2 aqueous solution with up to 28-fold improvement to 83 $\mu\text{g/mL}$, surpassing the aqueous solubility of all previously reported thiostrepton derivatives prepared by semisynthetic or biosynthetic methods.²

Results and Discussion

For $\text{C}(\text{sp}^2)\text{—H}$ functionalization of the Dhas of **1**, we evaluated a range of coupling partners, including isocyanates⁹ and electrophilic alkenes such as enones,⁸ acrylates, and maleimides.¹⁰ Unfortunately, under a variety of conditions and with different transition metal catalysts, little to no desired products were obtained, often with significant degradation of the natural product. Relevant to these studies, thiostrepton completely degraded in the presence of $\text{Cu}(\text{OAc})_2$ or AgOAc , stoichiometric oxidants that are commonly used in C—H functionalization reactions.

We then directed our attention to 1,4,2-dioxazolones as potential amidation reagents. Chang and coworkers initially demonstrated that these reagents are particularly effective coupling partners for C—H amidation,¹¹ and they have since been employed by many research labs for a range of different applications.¹² We hypothesized that due to the inherently polar character of amide functionality, selective C—H amidation of Dha $\text{C}(\text{sp}^2)\text{—H}$ bonds could provide new analogs of thiostrepton with improved aqueous solubility. Significantly, β -amidated Dhas are found in stable bioactive natural products, suggesting that the amidated thiostrepton analogs would also be stable.¹³

While $\text{C}(\text{sp}^2)\text{—H}$ amidation of Dha residues have not previously been reported, structurally, Dhas bear aspects of both acrylamides and enamides, motifs that have been modified by $\text{C}(\text{sp}^2)\text{—H}$ functionalization. Specifically, Li and co-workers had shown that *N*-methoxyacrylamides were viable substrates for C—H amidations with dioxazolones.¹²ⁱ Although the $\text{C}(\text{sp}^2)\text{—H}$ amidation of enamides has not been reported, we had previously found that enamides are effective substrates for other types of $\text{C}(\text{sp}^2)\text{—H}$ functionalization under mild conditions.¹⁴

An optimized reaction system with 10 equiv of 3-phenyl-1,4,2-dioxazolone and 50 mol % of $[\text{Cp}^*\text{Co}(\text{MeCN})_3][\text{SbF}_6]_2$ ¹⁵ proved to be effective for conversion of **1** to amidation products. A 71% overall yield of amidated derivatives of **1** was obtained in 9:1 solvent mixture of 1,2-DCE/TFE based on crude UPLC analysis (Figure 2B, entry 1, see Table 1).¹⁶ Dha1 was determined to be the predominant site for C—H amidation as determined by NMR analysis (see Supporting Information). A mixed solvent system was found to be important for the desired reactivity; neat fluorinated alcohols such as HFIP led to consumption of **1** without detection of products by UPLC (entry 2). Negligible conversions were observed with 1,2-DCE and 1,4-dioxane as solvents owing to insolubility of **1** and the Co catalyst in those solvents, respectively (entries 3-4). Other mixed solvent systems allowed for significant product formation, but in lower yields relative to the DCE/TFE system (compare entry 1 with entries 5-6).

Upon determination of optimal solvent conditions, we further evaluated the importance of other components of the reaction system. Minimal conversion to product occurred at 25 °C (Figure 2B, entry 7). No loss in yield was observed upon lowering the dioxazolone loading to 5 equivalents (entry 8), but the yield was reduced when 2 equivalents of the amidating agent was used (entry 9). Finally, the importance of the Co catalyst and its loading amount was established through use of an analogous Rh catalyst as well as a 10% loading of the Co catalyst, with both reactions showing negligible conversion of **1** (entries 10-11). Relatively high catalyst loading is needed likely due to sequestration by the plethora of functionalities within **1**; however, the use of an earth abundant cobalt catalyst diminishes concern about this higher loading, and product purification was unimpeded by the presence of somewhat higher catalyst concentration.

With effective C—H amidation conditions in hand, we next evaluated a broader set of dioxazolone substrates for derivatization of **1** (Scheme 1). In addition to the phenyl substituent used for reaction optimization (vide supra), anisole (**2b**) and halobenzene (**2c-d**) substituents were readily installed with moderate yields and regioselectivities. Although an inductively-withdrawing CF₃ group at the para position gave only trace amount of **2e**, electron withdrawing substituents at the meta-position of the phenyl ring were tolerated, as exemplified by analog **2f** with the meta-acetyl group. The method also enabled the introduction of nonaromatic amide groups; analogs containing alkyl chains (**2g-i**) and a tethered ester (**2j**) could be effectively synthesized with respectable yields and regioselectivities. Furthermore, MOM and MEM ethers (**2k-l**) were incorporated to give the respective ether-substituted amides with superb regioselectivities.

Having demonstrated the ability to synthesize analogs of **1** through mild, single-step C—H amidation, we then explored the potential of these analogs to serve as platforms for the modular introduction of additional functionality. Carbonyl groups are known handles for bioorthogonal oxime formation to enable a broad range of applications, including alteration of physicochemical properties and drug targeting.¹⁷ However, standard conditions for oxime formation from ketone-containing peptides¹⁷ caused extensive degradation of the thiostrepton derivative **2f** appended with a ketone. We therefore explored alternate strategies for oxime synthesis that might be more compatible with the delicate nature of thiostrepton.

We expected the greater reactivity of aldehydes relative to ketones to enable oxime formation under milder conditions, and to that end we synthesized dioxazolone **4**. Amidation of **1** with **4** generated crude mixtures that could be immediately used for oxime formation with an assortment of O-substituted hydroxylamines (Scheme 2). In addition to the synthesis of O-methyl oxime (**5a**) and hydroxime (**5b**) analogs, oxime tethering proved competent for the installation of various unprotected protic functionalities. Carboxylic acids (**5c-d**) and an alcohol (**5e**) were introduced in moderate yields over two steps, as determined by UPLC. A PEG₃ chain was also appended to thiostrepton by the amidation-condensation sequence (**5f**). Finally, synthesis of **5g** demonstrated that even unprotected glycosyl units could be introduced.

We next evaluated the effects of the pendant amide substituents at Dha1 on the aqueous solubility of thiostrepton derivatives (Table 1). The thermodynamic solubility of **1** was first

assessed in 10 mM aqueous MOPS buffer (pH 7.2) and was found to be $3.0 \pm 0.2 \mu\text{g/mL}$ (see Supplemental Information for details). Products **2g** and **2i-2l** each showed substantial improvements in solubility, with **2g** giving the highest solubility ($83 \pm 5 \mu\text{g/mL}$; 28-fold increase). The benzamide derivatives **2a**, **2c**, and **2e** showed poor solubilities below detection limits on UPLC, indicating that an aromatic ring was a poor fit for improving solubility as a key pharmacokinetic parameter, likely owing to an overall enhancement of lipophilic character relative to **1**. Although oxime derivatives **4a-b** and **4e** also showed an overall reduction in aqueous solubility, the polar groups of **4c** (carboxylic acid), **4f** (PEG), and **4g** (hexose) successfully compensated for the lipophilic aryl ring to effect substantial improvements in solubility relative to **1**.

Given the large size of thiostrepton, it is somewhat surprising that a wide range of solubilities were observed for the thiostrepton derivatives. The site-specific modification of a solvent-exposed Dha site was perhaps important for achieving pronounced effects on solubility. These results validate C—H amidation as a potential tool for improving physicochemical properties of bioactive molecules, such as solubility in the case of **1**.

Site-specific modification in the tail region of thiostrepton should also likely be beneficial for maintaining antibacterial activity. X-ray structural determination of thiostrepton bound to the 50S ribosomal subunit have established that the tail region is solvent exposed,¹⁸ with modifications to this region generally well tolerated.^{6b-d,7a} Analogs **2a-l** and **4a-g** were consequently evaluated for activity against a broad spectrum of Gram-positive bacteria (Table 2). Many analogs demonstrated activity within 2-5 times the MICs observed for **1** (Table 2, bolded entries). Importantly, except for **4g**, all compounds were generally competitive with vancomycin, a potent compound currently used for treating bacterial infections.¹⁹ Only VSE *E. faecium* showed consistent vulnerability to vancomycin over the derivatives of **1**. The most active analogs, including **2c**, **4a**, and **4c**, possessed relatively nonpolar amides. In contrast, compounds with the smallest alkyl (**2g-h**) or a polar carbohydrate group (**4g**) had the poorest activity.

Conclusion

We have disclosed the site-selective modification of Dha1 of the natural product thiostrepton (**1**) by Cp*-catalyzed C—H amidation with dioxazolone coupling partners. This approach, which represents the first example of C—H functionalization of Dha residues to maintain the alkene character of the Dha residue, enabled the preparation of a number of novel analogs of **1**. In addition, by using this approach to introduce a formyl group, the modular introduction of diverse functional groups by bioorthogonal oxime formation was also accomplished. Antibiotic activity was broadly maintained, and many of the derivatives showed greatly improved aqueous solubility. These results demonstrate the potential of Dha C(sp²)—H amidation for the synthesis of bioactive analogs of thiostrepton with improved physicochemical properties.

The current set of thiostrepton derivatives incorporate a variety of polar functionality, but none incorporate basic amines. Hergenrother and coworkers have recently reported that appending amines to antibiotics can result in dramatic improvements in accumulation and

cytotoxicity for Gram-negative bacteria along with beneficial effects for Gram-positive bacteria.²⁰ The preparation of derivatives of **1** that incorporate basic amines should improve aqueous solubility while maintaining and potentially improving activity against Gram-positive bacteria. These analogs could possibly even elicit activity against Gram-negative bacteria for which thiostrepton is inactive. The introduction of amine functionality will therefore be the focus of future work.

Beyond the immediate applications in thiostrepton derivatization, these first examples of Dha C(sp²)—H functionalization could feasibly be extended to other natural products as well as peptides and proteins that contain Dha residues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Support from the NIH is gratefully acknowledged, R35GM122473 to JAE and R35GM132092 to SJM.

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Previous work: Thiostrepton analogs by conjugate addition to Dhas

This work: Thiostrepton analogs by C(sp²)-H amidation

Figure 1.
Thiostrepton **1** and applicability of Dha residues as scaffolds for analog synthesis.

Figure 2.

Optimization of reaction conditions for thiostrepton C—H amidation. (a) Reaction scheme and generated regioisomers. (b) Conditions screened for C—H amidation. (c) Crude UPLC chromatogram for optimized conditions (entry 8).

UPLC yields relative to N-Boc aniline internal standard. Regomeric ratios determined by UPLC in parentheses. ^a1:1 DCE/dioxane solvent.

Scheme 1.
Scope of thioestrepton C—H amidation.

Scheme 2.

Oxime synthesis from a C—H amidation-condensation sequence.

Table 1.

Aqueous solubility of thiostrepton derivatives.

Entry	Compound	Solubility ($\mu\text{g/mL}$) ^[a]
1	1	3.0 ± 0.2
2	2a	<1.0
3	2c	<1.0
4	2e	1.1 ± 0.2
5	2g	83 ± 5
6	2h	<1.0
7	2i	16.2 ± 0.6
8	2j	22 ± 2
9	2k	28 ± 4
10	2l	19 ± 1
11	5a	<1.0
12	5b	<1.0
13	5c	20 ± 1
14	5e	1.4 ± 0.4
15	5f	4.3 ± 0.4
16	5g	11 ± 1

Bolded entries indicate improvements in solubility relative to **1**.^[a] Aqueous concentration after 24 h stirring in 10 mM aqueous MOPS buffer (average of two measurements).

Table 2.

Antibacterial activity of **1** and derivatives.

Compound	MIC (µg/mL) for Gram-positive organisms									
	<i>S. aureus</i> (MSSA)	<i>S. aureus</i> (MRSA)	<i>E. faecalis</i> (VSE)	<i>E. faecalis</i> (VRE)	<i>E. faecium</i> (VSE)	<i>E. faecium</i> (VRE)	<i>S. pneumoniae</i>	<i>S. pyogenes</i>		
1	0.06	0.12	0.12	0.12	0.12	0.12	<0.001	<0.001		
2b	1	2	1	1	0.5	0.5	0.008	0.002		
2b	1	2	2	1	1	1	0.004	0.004		
2c	0.25	0.5	0.25	0.25	0.25	0.12	<0.001	0.002		
2d	1	1	0.5	0.5	0.5	0.5	<0.001	<0.001		
2f	1	2	1	1	1	0.5	0.004	0.004		
2g	2	4	4	4	4	2	0.06	0.06		
2h	2	4	4	4	4	2	0.06	0.06		
2i	0.5	0.5	0.5	1	1	0.25	0.004	0.004		
2k	0.5	1	1	1	1	0.5	0.015	0.008		
2l	1	1	1	1	1	1	0.015	0.015		
5a	0.5	0.5	0.5	0.25	0.25	0.25	<0.001	<0.001		
5b	0.5	0.5	1	0.5	0.5	0.5	0.002	0.004		
5c	1	1	2	1	2	1	0.015	0.015		
5d	1	1	2	1	1	1	0.015	0.015		
5e	0.5	0.5	0.5	0.5	1	0.5	0.004	0.004		
5f	0.5	1	1	0.5	1	0.5	0.002	0.004		
5g	16	16	16	16	16	8	0.25	0.25		
vancomycin	1	1	2	>32	0.25	>32	0.12	0.25		