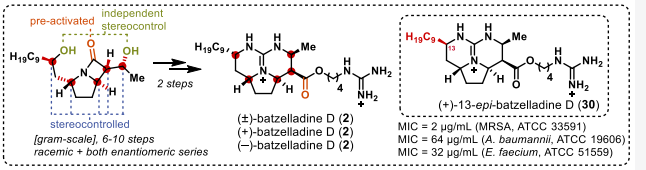
JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Article

Concise Synthesis and Antimicrobial Evaluation of the Guanidinium Alkaloid Batzelladine D: Development of a Stereodivergent Strategy

You-Chen Lin, Aubert Ribaucourt, Yasamin Moazami, and Joshua G. Pierce*

Cite This: https://dx.doi.org/10.1021/jacs.0c04091
Read Online

ABSTRACT: Herein, we describe a stereodivergent route to (±)-batzelladine D (**2**), (+)-batzelladine D (**2**), (−)-batzelladine D (**2**), and a series of stereochemical analogues and explore their antimicrobial activity for the first time. The concise synthetic approach enables access to the natural products in a sequence of 8−12 steps from readily available building blocks. Highlights of the synthetic strategy include gram-scale preparation of a late stage intermediate, pinpoint stereocontrol around the tricyclic skeleton, and a modular strategy that enables analogue generation. A key bicyclic β-lactam intermediate not only serves as the key controlling element for pyrrolidine stereochemistry but also serves as a preactivated coupling partner to install the ester side chain. The stereocontrolled synthesis allowed for the investigation of the antimicrobial activity of batzelladine D, demonstrating promising activity that is more potent for non-natural stereoisomers.



INTRODUCTION

The marine environment is one of the most prolific sources of chemically complex and biologically active molecular scaffolds such as polycyclic guanidinium alkaloids (PGAs).[1,2] Since first isolated in 1989, PGAs have been studied extensively, revealing valuable information regarding their biosynthetic pathways and molecular properties.[3−6] Despite these significant contributions, surprisingly little information regarding PGAs biological function and targets is known for most classes of PGAs. Efficient and modular synthetic approaches to these molecular scaffolds allow not only for comprehensive biological studies but also for the generation of derivatives with systematic stereochemical and functional group modifications.

The batzelladines are a family of PGAs that were isolated in the mid-1990s from the Caribbean sponge *batzella* sp.[7] This family of molecules possess a tricyclic guanidinium core bearing a guanidine-functionalized side chain of varying complexity as highlighted by batzelladine A (**1**), B (**3**), and D (**2**) (Figure 1a).[7−11] The guanidinium core of the batzelladines bears a pyrrolidine motif that is either of the *cis*- or *trans*-stereoconfiguration anchored to the various side chains through an ester linkage. The structural complexity of these scaffolds, in addition to antiviral and cytotoxicity activity reported by the isolation team, has led to significant interest from the synthetic community. *Cis*-pyrrolidine members of the batzelladine family, represented by batzelladine B (**3**), were first to draw the attention of the synthetic community,[12−16] with **3** itself succumbing to an elegant approach from Herzon and co-workers in 2015.[17,18] The *trans*-pyrrolidine bearing family members, highlighted by batzelladine A (**1**) and D (**2**) represent distinct synthetic challenges. The first synthesis of a member of this subfamily was batzelladine D (**2**) by Overman and co-workers through the use of a tethered Biginelli strategy,[19−21] followed by a 1,3-dipolar cycloaddition approach by Nagasawa and co-workers.[22,23] More recent efforts by Gin and Evans provide a [4 + 2] and radical cyclization approaches to the *trans*-batzelladine core, respectively.[24,25]

From a biological point of view, the batzelladines have received attention due to their reported activity as inhibitors of HIV gp120-human CD4 binding.[4,5,7−11,22,26] These seminal studies revealed the significance of the batzelladine side chain on activity, with batzelladines A (**1**) and B (**3**) active in the protein−protein interaction assay while batzelladine D (**2**) was inactive. Subsequent studies by Nagasawa have suggested batzelladine D (**2**) does indeed bind to CD4,[22] but a comprehensive biological examination of this subfamily of natural products is lacking, particularly for members bearing simplified side chains such as **2**.[5] Regardless of their potential as therapeutic agents, these scaffolds represent exciting platforms for the generation of chemical probes to study protein−protein interactions as well as to explore the broader biological activities of these PGAs.

RESULTS AND DISCUSSION

Retrosynthetic Analysis. As part of a broader program targeting modular and practical syntheses of PGAs,[5,27] we became interested in developing a synthesis of batzelladine D (**2**) that would facilitate access to an array of stereochemical

Received: April 15, 2020

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

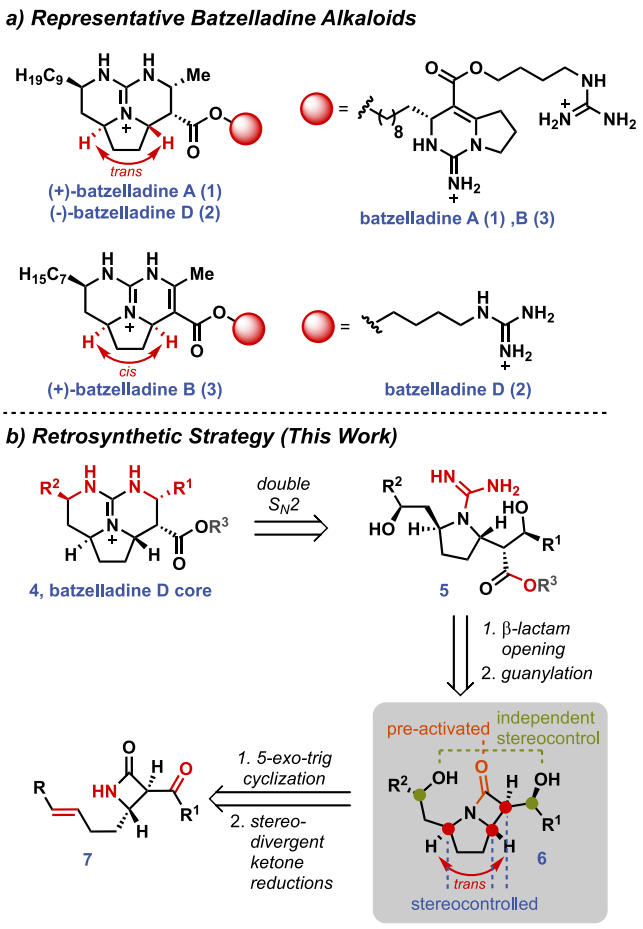
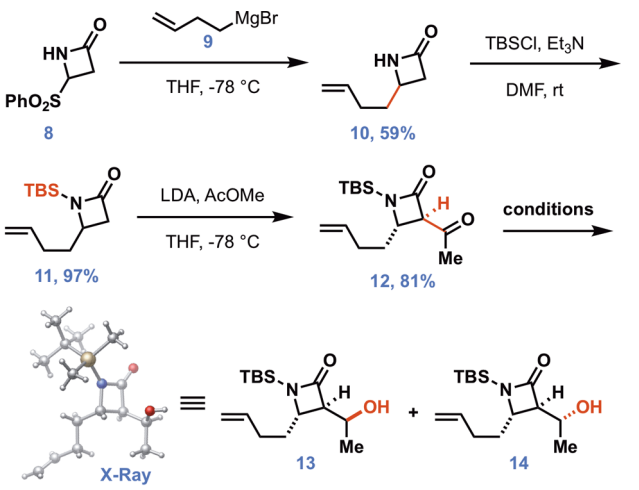


Figure 1. (a) Representative batzelladine alkaloids; (b) retrosynthetic strategy presented herein.

and functional derivatives to enable expanded biological studies. To this end, our synthetic strategy envisioned the formation of the tricyclic guanidinium core **4** through a double S_N2 reaction of a suitably functionalized guanylated diol precursor **5** (Figure 1b). Such a strategy was inspired by previous efforts but represents a one-pot solution that should allow for the generation of all possible stereoisomers from a common intermediate.[19,23] Access to the fully functionalized *trans*-pyrrolidine relies on the use of a key bicyclic *β*-lactam intermediate (**6**, Figure 1b) that would serve not only as a preactivated coupling partner though the lactam itself but also to control the stereochemistry of the pyrrolidine core. **6** would be prepared through an aza-Michael addition of monocyclic *β*-lactam **7**, controlling the pyrrolidine stereochemistry while allowing independent control both alcohols through diastereoselective ketone reductions. Importantly, the *β*-lactam approach allows for the thermodynamic installation and retention of the labile 1,3-dicarbonyl stereochemistry and can be accessed through an array of well-established synthetic methods.[28−31] This approach is distinct from previous work which utilizes *β*-lactams as starting materials,[32] as the *β*-lactam herein provides a late-state intermediate required for stereocontrol and carbonyl activation. Most importantly, the *β*-lactam enables the installation of the trans-pyrrolidine core: inverting the *cis*-selectivity observed for typical aza-Michael reactions and that of elegant routes toward the *cis*-pyrrolidine containing batzelladine families.[12,13]

Development of Stereodivergent Access to *β*-lactam Building Blocks. To begin exploring the possibility of utilizing a series of enantiomeric and diastereomeric *β*-lactam building blocks toward the batzelladines, we targeted a racemic synthesis of a compound such as **7** (Figure 1b). Our initial efforts toward this goal began with commercially available *β*-lactam **8** which was substituted with butenyl Grignard reagent **9**,[33] *N*-protected with TBS and subsequently acylated to provide **12** as a single diastereomer in good yield over 3 steps (Figure 2). At this stage, reduction of the methyl ketone was



entry[a]	conditions	results
1	Li(*t*-BuO)(*i*-Bu)$_2$Al-H, -78 °C, 0.5 h	40% with 2:1 dr
2	DIBAL, -78 °C, 0.5 h	30% with 3:1 dr
3[b]	K-selectride + KI, 25 °C, 1 h	55% with 5:1 dr
4[b]	K-selectride + KBF_4, 0 °C, 0.5 h	80% with 10:1 dr
5	$NaBH_4$, 0 °C, 2 h	36% with 1:1 dr
6	L-selectride, -78 °C, 0.5 h	40% with 1:5 dr
7	$Zn(BH_4)_2$ (1 equiv), 0 °C, 1 h	35% with 2:5 dr
8	Li(*t*-BuO)$_3$Al-H, -78 °C, 2 h	40% with 2:5 dr
9	Li(Ph)(*i*-Bu)$_2$Al-H, -78 °C, 0.5 h	24% with 1:3 dr
10	Li(*sec*-Bu)(*i*-Bu)$_2$Al-H, -78 °C, 0.5 h	25% with 1:4 dr
11	Li(*n*-Bu)(*i*-Bu)$_2$Al-H, -78 °C, 0.5 h	64% with 1:10 dr

[a]All reactions were perfomed at 0.1 M in THF and 1.2 equiv reductant were used, unless otherwise noted. [b]Reactions was perfomed in 0.1 M Et_2O and 1.2 equiv *additive was used.*

Figure 2. Synthesis of *β*-lactam building blocks **13** and **14**.

required, and we sought to develop conditions to access both diastereomers, ultimately enabling both natural product epimers via subsequent displacement (Figure 1b). After screening a variety of reduction conditions (Figure 2), we found that K-selectride/KBF_4 in ether provided the desired diastereomer **13** in 10:1 dr and 80% yield (entry 4) with the stereochemistry confirmed through X-ray analysis while Li(*n*-Bu)(*i*-Bu)$_2$Al−H provided the opposite diastereomer **14** required for batzelladine D (**2**) in 10:1 dr and 64% yield (entry 11).[34]

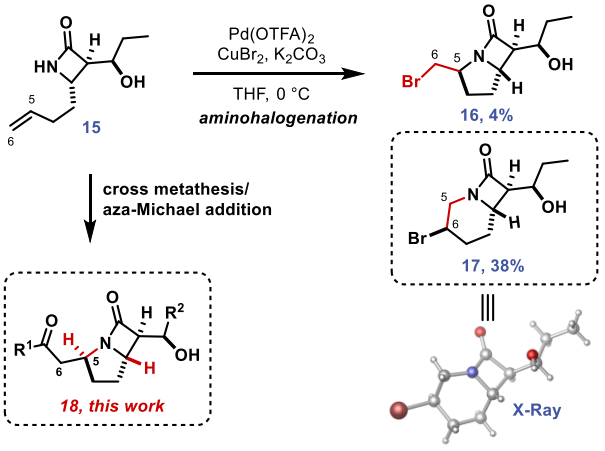
In pursuing these conditions, we were motivated to explore additives that could enhance selectivity and fortunately found that a potassium counterion[35] and KBF_4 additive were critical to form the desired reduction and promote reactivity, while that selectivity could be inverted though the use of bulky aluminum hydride reagents. Further studies are required to

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

uncover the mechanistic underpinnings of this selectivity enhancement, but this reagent combination may prove useful in other directed diastereoselective reductions.

Cyclization Approaches to Form Bicyclic β-Lactams. Bicyclic β-lactams are common scaffolds in antimicrobial natural products but have seen limited utility as strategic building blocks for synthesis.[36−38] As highlighted in our retrosynthetic approach, we require a [4.5]-fused bicyclic β-lactam **6** (Figure 1b) bearing a trans-pyrrolidine core. Straightforward methods to construct such functionalized scaffolds are not currently available,[38−44] and we therefore embarked on studies to append the requisite ring to β-lactams such as **15**. A number of approaches including amino-halogenation,[45] aza-Heck,[46−48] amination/cross-coupling,[49−51] and carbonylative amidation[52,53] resulted in no cyclization but instead direct intermolecular reactivity (see SI for details). These results provided an initial glimpse into the strained nature of these [4.5]-fused bicyclic β-lactams. To highlight these challenges, we found that Pd-catalyzed aminohalogenation proceeded under standard reaction conditions,[54] but to our surprise, instead of obtaining **16**, the reaction provided almost exclusively the [4.6]-fused bicycle **17** (Scheme 1). In

Scheme 1. Strategies to Prepare Bicyclic β-Lactams



parallel to these studies, we were also exploring approaches in which the alkene was prefunctionalized to promote our desired 5-membered ring formation and targeted a cross-metathesis/aza-Michael sequence. The feasibility of this approach was uncertain as these reactions have proven highly reversible in the literature and the strained nature of the bicyclic β-lactam **18** may prove problematic in that regard.[15,55]

To explore the potential of this approach, we prepared the functionalized model β-lactam **19** (see SI for details, Table 1). We initially utilized substrate **19** for screening that bears an ethyl side chain due to the straightforward nature of its synthesis via an aldol reaction with propionaldehyde, but subsequently confirmed our successful reactions on the methyl derivative required for **2**. With **19** in hand, we began evaluating conditions to promote the desired aza-Michael reaction[56−59] to generate **20**. Initially, a variety of Lewis acid and Bronsted acids were explored with no reaction or complex mixtures observed, respectively (entries 1−6, Table 1). Turning our attention to basic conditions, a number of bases including potassium tert-butoxide, LDA and sodium hydride did not prove successful (entries 7−12, Table 1); however, upon

Table 1. Aza-Michael Optimization Model Study

entry[a]	conditions	results[b]
1	$ZrCl_4$ (20 mol %), CH_2Cl_2, 25 °C	NR
2	AuCl (10 mol %), toluene, 110 °C	NR
3	$PtCl_4$ (10 mol %), CH_2Cl_2, 25 °C	NR
4	$ReCl_5$ (10 mol %), CH_2Cl_2, 25 °C	NR
5	$BF_3{\cdot}Et_2O$ (20 mol %), CH_2Cl_2, 25 °C	NR
6	HCl (50 mol %), Et_2O, 25 °C	complex mixture
7	K-*t*-butoxide (2 equiv), THF, −78 °C	complex mixture
8	K-*t*-butoxide (5 equiv), THF, 45 °C	trace product
9	TBAH (0.2 equiv)/THF, 25 °C	NR
10	LDA (2 equiv), THF, −78 °C	complex mixture
11	EtMgBr (2 equiv), THF, 0 °C	NR
12	NaH (2.2 equiv), THF, 0 °C	NR
13	LiHMDS (2.2 equiv), 25 °C	trace product
14[b]	LiHMDS (1.2equiv), 25 °C	39%[c]
15[b]	LiHMDS (0.7 equiv), 25 °C	89%[c]
16[b]	KHMDS (0.7 equiv), 25 °C	70%[c]

[a]All reactions were monitored every 0.1, 0.5, 1, 3, 6, 16 h unless otherwise noted. [b]Reactions were quenched after 10 min. [c]>10:1 *trans* to *cis* diastereoselectivity. [d]NR = no reaction.

exploration of substoichiometric amount of lithium or potassium bis(trimethylsilyl)amide bases, we were gratified to observe high levels of conversion and yield to generate our desired [4.5]-fused bicyclic β-lactam **20** (entries 13−16, Table 1).[60] Further, we were able to confirm the reaction proceeded to provide the *trans*-pyrrolidine stereochemistry in >10:1 dr as confirmed by NOSEY experiments (see SI for details).

The diastereoselectivity of this process is significant, as the β-lactam aza-Michael addition provides the *trans*-pyrrolidine core, whereas previous aza-Michael addition strategies provide the *cis*-pyrrolidine.[12,13] The profound differences observed by altering the stoichiometry and identify of the base employed points to a reversible reaction due to the added ring strain of the bicyclic system and the potential instability of the reaction product upon prolonged exposure to strong base. In attempts to equilibrate the system to the *cis*-pyrrolidine, only increasing decomposition is observed with increases in base, temperature, or time.

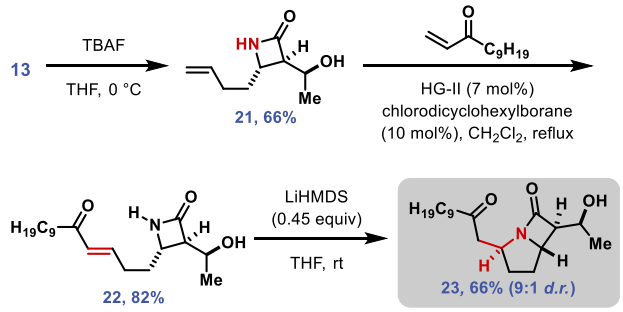
Stereoselective Ketone Reduction. With our desired bicycle in hand, we were in a position to explore the last two hurdles to preparing the targeted linear precursor **5** that would undergo cyclization to form the tricyclic guanidinium scaffold (Figure 1b). First, conditions needed to be identified for mild β-lactam ring opening to install the ester side chain required for the natural products. Second, conditions needed to be identified for selective reduction to both diastereomers of the remaining ketone, as this will allow for both natural product epimers to be generated from a common intermediate (as was possible for the other alcohol; see Figure 2). Our initial efforts were focused on conducting the β-lactam ring opening under a variety of acidic and basic conditions; surprisingly, this reaction proved challenging with most conditions providing low levels of reactivity (see SI for details). The best conditions identified were use of boron Lewis acids in dichloromethane, but even in these cases, the reaction proved challenging to reproduce.

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

We hypothesized that the challenges could be due to the presence of the ketone and potential retro-aza-Michael reactions and/or other reaction pathways, and selected to conduct ketone reduction. This rationale was confirmed by reducing the ketone on test scale with $NaBH_4$ and then subsequently opening the β-lactam with $BF_3{\cdot}OEt_2$, which proceeded smoothly by TLC and gave us confidence to delay the lactam opening step to after stereoselective ketone reduction.

To evaluate the conditions required for stereoselective ketone reduction, bicyclic β-lactam **23** was prepared smoothly via a three step TBS deprotection, cross-metathesis,[61] and aza-Michael cyclization sequence (Scheme 2). It is worth noting

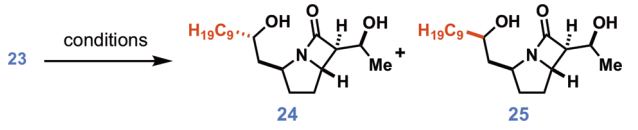
Scheme 2. Preparation of Bicyclic β-Lactam 23



that the *aza*-Michael cyclization proceeds smoothly in the presence of the free hydroxyl group, whereas in our model, we employed a TBS protecting group. With ample quantities of **23** in hand, an array of conditions was screened (Table 2). Most reaction conditions proceeded to provide the opposite diastereomer **25** required for batzelladine, with Luche conditions providing 97% yield and 6:1 dr (entry 7, Table 2). Fortunately, moving to bulky hydride reagents began to invert this selectivity, and we ultimately identified K-selectride in toluene as the optimal conditions yielding **24** in 68% yield and a 3:5 dr favoring the desired diastereomer (entry 14, Table 2). While this dr is modest, the reaction is highly scalable and provides preparatively useful quantities of material that can be separated and recycled if desired. Initial attempts at employing chiral reagents to overcome this selectivity were unsuccessful (entry 5, Table 2), but further studies are underway to explore additional systems in this regard. Regardless, the optimized conditions provide selective access to the unnatural diastereomer and practically useful access to the natural stereoisomer.

Endgame and Synthesis of (±)-Batzelladine D. At this stage, having access to all 4-alcohol diastereomers, we were positioned to optimize the end game of our synthetic approach and gain access to (±)-batzelladine D, (±)-13-epi-batzelladine D, and (±)-15-epi-batzelladine D. As shown in Scheme 3, opening of β-lactams **24** and **25** with side chain **26** proceed smoothly upon activation with $BF_3{\cdot}OEt$ to provide the target dihydroxy pyrrolidines **27** and **28**. Installation of the guanidine by treatment with *N*,*N*-di-Boc-*S*-methylisothiourea and mercury chloride[62] was followed in the same pot by mesylation of both alcohols, rapid displacement by the guanidine to install the tricyclic core, and final treatment with formic acid to cleave both the core and side-chain Boc protecting groups. This one-pot process installed the three critical bonds of the core in a stereoselective fashion and provided a separable 1.0:1.2 mixture of (±)-batzelladine D (**2**) and **29**, resulting from β-mesylate elimination under the reaction conditions with 41%

Table 2. Selected Reduction Attempts on Ketone 17



entry[a]	conditions	results[e] 24:25
1	DIBAL (0.75equiv), THF, −78 °C to rt, 15 h	SM observed[b]
2	$LiBH_4$ (1.1 equiv), THF, −78 °C, 3 h	24% with 1:1 dr[b]
3	$Li(t\text{-}BuO)_3Al{-}H$ (1.2 equiv), THF, −78 °C, 0.5 h	50% with 5:4 dr
4	$Zn(BH_4)_2$ (1 equiv), THF, −3 °C, 12 h	77% with 1:3 dr
5	CBS (100 mol %), BMS (2 equiv) CH_2Cl_2, 0 °C, 5 min	30% 1:1 dr[b,c]
6	$CeCl_3$ (20 mol %), $NaBH_4$ (2 equiv) MeOH, −25 °C, 1 h	50% 1:4 dr
7	$CeCl_3$ (20 mol %), $NaBH_4$ (2 equiv) MeOH, −78 °C, 5 min	97% 1:6 dr
8	L-selectride (1.2 equiv), THF, −78 °C, 30 min	45% 1:5 dr
9	K-selectride (1.2 equiv), THF, −78 °C, 30 min	60% with 1:1 dr
10	K-selectride (1.2 equiv), Et_2O, −78 °C, 30 min	40% 4:3 dr
11	K-selectride (1.2 equiv), Et_2O, 25 °C, 3 min	45% 3:2 dr
12	K-selectride (1.03 equiv), KBF_4 (1.2 equiv) Et_2O, 25 °C, 3 min	64% 5:4 dr
13	K-selectride (0.97 equiv), KBF_4 (1.2 equiv) 0.01 M Et_2O, 25 °C, 3 min	59% 5:4 dr
14	K-selectride (1.15 equiv), KBF_4 (1.2 equiv) 0.01 M toluene, 25 °C, 3 min	68% 5:3 dr
15	$(PPh_3)_3RhCl$ 10 mol %, H_2 balloon EtOAc, 25 °C, 24 h	NR
16[d]	Pt/C cartridge, 120 bar H_2 EtOAc, 55 °C, 1 h	NR
17[d]	Ru/C cartridge, 120 bar H_2 EtOAc, 55 °C, 1 h	60% 1:1 dr

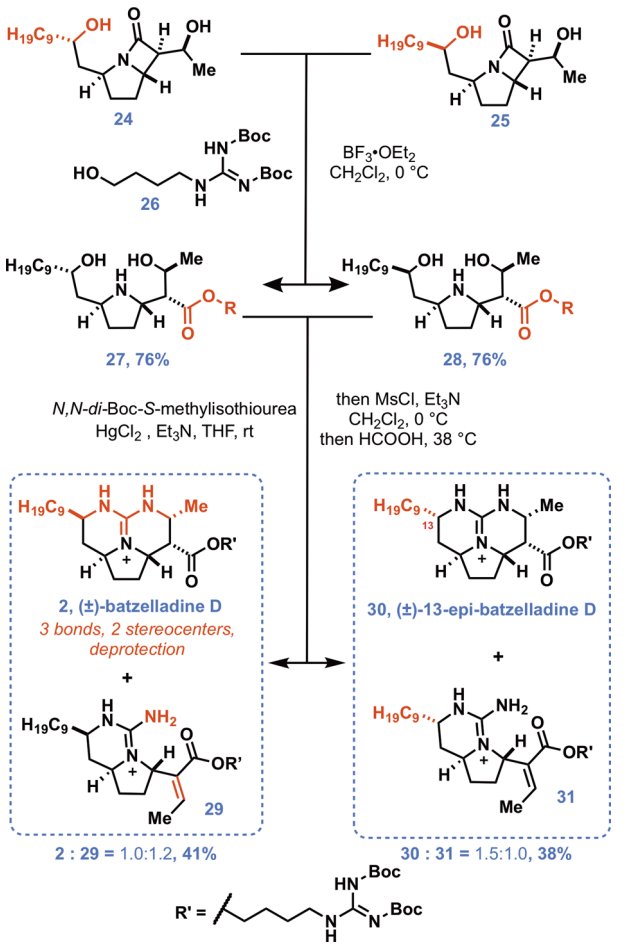
[a]All reactions were performed at 0.1 M, unless otherwise noted. [b]Over reduced product, $[M + H]^+ = 326$ dominated. [c]Enantiopure starting material was used. [d]Reaction was performed in H-cube at 0.05 M, 1 mL/min. [e]Isolated yield.

overall yield. Utilizing this endgame, (±)-13-epi-batzelladine D (**30**) and **31** were prepared from **28** (Scheme 2) and (±)-15-epi-batzelladine D (**S1**) was also prepared from the corresponding alcohol epimer **14** (See SI for details). This final reaction cascade is plagued by the formation of the elimination byproducts, and we have spent considerable effort attempting to optimize this process (see Table S9−S10 for full reaction screening). Overall, it does not appear that a simple E1CB reaction accounts for the outcomes observed, and we have been unable to significantly improve the product ratios to date. Given the straightforward reaction conditions and our ability to readily separate the product from the elimination product, we have elected to utilize this optimized protocol to access the enantioenriched natural products and their stereochemical analogues.

Asymmetric Synthesis of (+)-Batzelladine D and (−)-Batzelladine D. Having a concise synthesis of (±)-batzelladine D (**2**) in hand, along with access to diastereomers of the natural product, we sought to also explore the generation of the enantiomeric series of compounds along with the generation of gram-scale quantities of our key intermediates (Scheme 4). To access the non-natural enantiomer, we chose to start from commercially available β-lactam **32**, already bearing the necessary hydroxyethyl side chain and available on

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

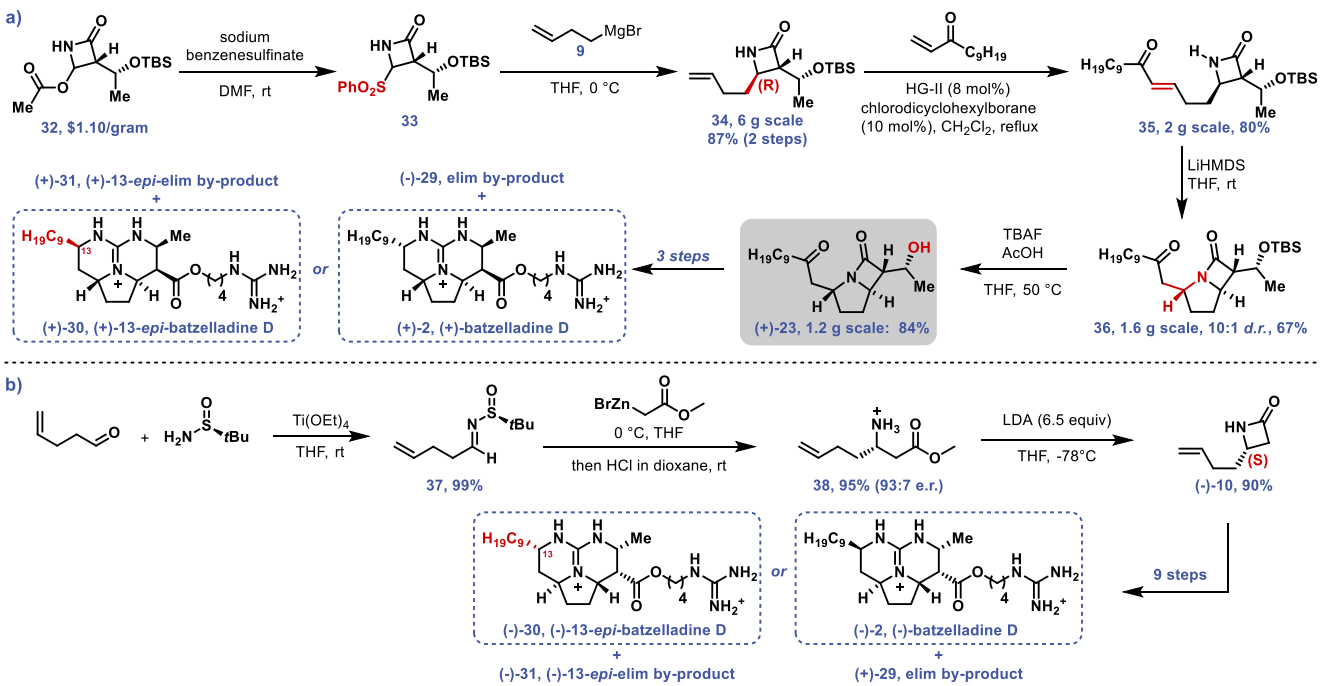
Scheme 3. Completion of the Racemic Synthesis of 2 and 30



large scale due to its use in antibiotic synthesis.[63] **32** could be readily converted to the requisite sulfone **33** through treatment with sodium benzenesulfinate and subsequently displaced by butenyl Grignard reagent **9** to generate **34** on 6-g scale (Scheme 3a). Cross-metathesis, aza-Michael addition, and TBS deprotection proceed smoothly, as before, to provide (+)-bicyclic β-lactam **23** on gram-scale, set to undergo diastereoselective reduction and conversion to (+)-batzelladine D (**2**) and (+)-13-epi-batzelladine D (**30**) as previously developed. The natural enantiomer required denovo synthesis, and we relied on an Ellman auxiliary approach that readily provided (−)-β-lactam **10** in three steps (Scheme 3b). This material of the natural enantiomeric series was advanced to (−)-batzelladine D (**2**) and (−)-13-epi-batzelladine D (**30**) through a 9-step sequence as previously described for racemic β-lactam **10**.

Initial Antimicrobial Evaluation. In addition to interest in having access to a stereochemical library of batzelladine analogues to study their reported activity as inhibitors of HIV gp120-human CD4 binding (ongoing collaborative efforts), we sought to explore their antimicrobial activity against a series of ESKAPE pathogens[64−66] (Table 3). In initial screening, (±)-batzelladine D (**2**) proved moderately active against both methicillin sensitive *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA), with an MIC of 8 μg/mL. This represents the first evaluation of batzelladine D's antimicrobial properties and gave us reason to evaluate our stereoisomer library, elimination byproducts, and synthetic intermediates for their antimicrobial activity against an expanded panel of bacterial pathogens. Upon systematic evaluation of the stereoisomers against MSSA and MRSA, it was revealed that non-natural stereoisomers were more active than the racemic natural product or natural enantiomeric series. This effect can be highlighted by comparison of non-natural (+)-13-epi-batzelladine D (**30**) and natural (−)-13-epi-batzelladine D (**30**), wherein the unnatural enantiomer is somewhat more active (Table 3). The effect of stereochemistry on antimicrobial activity suggests there may be distinct targets or pathways

Scheme 4. (a) Synthesis of (+)-Batzelladine D and Gram-Scale Access to β-Lactam 37 and (b) Synthesis of (−)-Batzelladine D



https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Table 3. Antimicrobial Evaluation MIC values (ug/mL) of Batzelladine D and Stereochemical Analogues

Strain / Compound	(±)-2[a]	(+)-2[b]	(-)-2[c]	(±)-30[d]	(+)-30[e]	(-)-30[f]	(±)-S1[g]	(±)-31[h]	(+)-31[i]	(-)-31[j]	(-)-29[k]	(+)-29[l]	(±)-17[m]	(±)-23[n]	(+)-23[o]	LZD[p]	CPL[q]	CST[r]
S. aureus 33591 (MRSA)	8	8	4	8	2	16	8	2	2	8	4	8	ND	128	64	1	ND	ND
S. aureus 29213 (MSSA)	8	8	8	8	2	8	8	2	2	8	4	8	>128	64	64	2	ND	ND
E. faecium 51559	ND	128	32	ND	32	32	ND	ND	16	ND	ND	64	ND	ND	ND	ND	8	ND
P. aeruginosa PAO1	ND	128	32	ND	32	64	ND	ND	32	ND	ND	128	ND	ND	ND	>33	ND	0.5
A. baumannii 19606	128	64	64	256	64	64	256	32	32	64	32	32	ND	> 128	> 128	64	ND	ND

[a]racemic batzelladine D. [b]enantiopure batzelladine D (non-natural). [c]enantiopure batzelladine D (natural). [d]racemic 13-epi-batzelladine D. [e]enantiopure 13-epi-batzelladine D (non-natural). [f]enantiopure 13-epi-batzelladine D (natural). [g]racemic 15-epi-batzelladine D. [h]racemic 13-epi-elim byproduct. [i]enantiopure 13-epi-elim byproduct (non-natural). [j]enantiopure 13-epi-elim byproduct (natural). [k]enantiopure elimination byproduct (non-natural). [l]enantiopure elimination byproduct (natural). [m]racemic [4.6]-fused bicyclic β-lactam. [n]racemic [4.5]-fused bicyclic β-lactam. [o]enantiopure [4.5]-fused bicyclic β-lactam. [p]LZD = linezolid (control). [q]CPL = Chloramphenicol (control). [r]Colistin = CST (control).

involved in the observed activity and warrants additional studies to enable a more in-depth evaluation. Further studies on expanded pathogens will also allow determination as to the significance of these findings more broadly. In addition to the natural products and their stereoisomers, elimination by-products (±)-**31** and (+)-**31** also showed activity against MSSA and MRSA, although these compounds may function by an alternate mechanism given their reactive Michael acceptor. Expanding the screening to Gram-negative pathogens revealed promising activity, particularly for (+)-**30** and (−)-**2**, with MICs of 32−64 μg/mL. While these are not clinically relevant potencies against these challenging pathogens, they provide a starting point for further optimization.

CONCLUSIONS

In conclusion, we have developed a platform for the synthesis of a variety of stereochemical isomers of the batzelladine core and have utilized this approach to prepare (+)- and (−) batzelladine D and a panel of selectively prepared diastereomeric isomers. Our approach utilizes β-lactam building blocks as key starting materials but more significantly, takes advantage of [4.5]-fused bicyclic β-lactams as key intermediates that enable stereocontrol of the core and provide preactivation of side chain coupling. This strategy effectively relays the single stereoisomer in the readily available β-lactam starting material to control the four additional stereocenters in the natural product. Access to batzelladine D (**2**) and stereoisomers allowed for the evaluation of these scaffolds as antimicrobial agents, revealing non-natural isomers with promising levels of activity. Although it is likely such molecules have multiple targets and mechanisms of action, further understanding of these molecules in a variety of biological systems may open the door to the identification of new targets and pathways for small molecule targeting by these and other classes of molecules.[67]

Future efforts are focused on an expanded analogue library to explore the SAR of this family, the synthesis of batzelladine A for study and comparison of HIV gp120-human CD4 binding, and the further study of the scope and mechanism of these molecules as antimicrobial agents. These investigations will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c04091.

Detailed experimental procedures, spectroscopic data, and ^{1}H and ^{13}C NMR spectra (PDF)

Crystallographic data for **rds852** (CIF)

Crystallographic data for **rds677** (CIF)

AUTHOR INFORMATION

Corresponding Author

Joshua G. Pierce − *Department of Chemistry, College of Sciences and Comparative Medicine Institute, NC State University, Raleigh, North Carolina 27695, United States;* orcid.org/0000-0001-9194-3765; Email: jgpierce@ncsu.edu

Authors

You-Chen Lin − *Department of Chemistry, College of Sciences and Comparative Medicine Institute, NC State University, Raleigh, North Carolina 27695, United States*

Aubert Ribaucourt − *Department of Chemistry, College of Sciences, NC State University, Raleigh, North Carolina 27695, United States*

Yasamin Moazami − *Department of Chemistry, College of Sciences, NC State University, Raleigh, North Carolina 27695, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.0c04091

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to the NIH (R01GM117570) for generous support of this work and to NC State University for support of our program. Mass spectrometry data, NMR data, and X-ray data were obtained at the NC State Molecular, Education, Technology and Research Innovation Center (METRIC). Kaylib Robinson and Christina Martinez-Brokaw are acknowledged for the training to conduct the antimicrobial assays. The S. aureus strains in Table 3, were provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH.

REFERENCES

(1) Sfecci, E.; Lacour, T.; Amade, P.; Mehiri, M. Polycyclic Guanidine Alkaloids from Poecilosclerida Marine Sponges. *Mar. Drugs* **2016**, *14* (4), 77.

(2) El-Demerdash, A.; Tammam, M. A.; Atanasov, A. G.; Hooper, J. N. A.; Al-Mourabit, A.; Kijjoa, A. Chemistry and Biological Activities

of the Marine Sponges of the Genera Mycale (Arenochalina), Biemna and Clathria. *Mar. Drugs* **2018**, *16* (6), 214.
(3) El-Demerdash, A.; Atanasov, A. G.; Bishayee, A.; Abdel-Mogib, M.; Hooper, J. N. A.; Al-Mourabit, A. Batzella, Crambe and Monanchora: Highly Prolific Marine Sponge Genera Yielding Compounds with Potential Applications for Cancer and Other Therapeutic Areas. *Nutrients* **2018**, *10* (1), 33.
(4) Liu, J.; Li, X.-W.; Guo, Y.-W. Recent Advances in the Isolation, Synthesis and Biological Activity of Marine Guanidine Alkaloids. *Mar. Drugs* **2017**, *15* (10), 324.
(5) Shi, Y.; Moazami, Y.; Pierce, J. G. Structure, Synthesis and Biological Properties of the Pentacyclic Guanidinium Alkaloids. *Bioorg. Med. Chem.* **2017**, *25* (11), 2817−2824.
(6) Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. Ptilomycalin A: A Novel Polycyclic Guanidine Alkaloid of Marine Origin. *J. Am. Chem. Soc.* **1989**, *111* (24), 8925−8926.
(7) Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; Brosse, C. D.; Mai, S.; Truneh, A.; Carte, B. Novel Alkaloids from the Sponge Batzella Sp.: Inhibitors of HIV Gp120-Human CD4 Binding. *J. Org. Chem.* **1995**, *60* (5), 1182−1188.
(8) Laville, R.; Thomas, O. P.; Berrué, F.; Marquez, D.; Vacelet, J.; Amade, P. Bioactive Guanidine Alkaloids from Two Caribbean Marine Sponges. *J. Nat. Prod.* **2009**, *72* (9), 1589−1594.
(9) Patil, A. D.; Freyer, A. J.; Taylor, P. B.; Carté, B.; Zuber, G.; Johnson, R. K.; Faulkner, D. J. Batzelladines F-I, Novel Alkaloids from the Sponge Batzella Sp.: Inducers of P56 Lck -CD4 Dissociation. *J. Org. Chem.* **1997**, *62* (6), 1814−1819.
(10) Hua, H.-M.; Peng, J.; Dunbar, D. C.; Schinazi, R. F.; Andrews, A. G. de C.; Cuevas, C.; Garcia-Fernandez, L. F.; Kelly, M.; Hamann, M. T. Batzelladine Alkaloids from the Caribbean Sponge Monanchora Unguifera and the Significant Activities against HIV-1 and AIDS Opportunistic Infectious Pathogens. *Tetrahedron* **2007**, *63* (45), 11179−11188.
(11) Gallimore, W. A.; Kelly, M.; Scheuer, P. J. Alkaloids from the Sponge Monanchora u Nguifera. *J. Nat. Prod.* **2005**, *68* (9), 1420−1423.
(12) Black, G. P.; Murphy, P. J.; Walshe, N. D. A. A Short Synthetic Route to the Tricyclic Guanidinium Core of the Batzelladine Alkaloids. *Tetrahedron* **1998**, *54* (32), 9481−9488.
(13) Black, G. P.; Murphy, P. J.; Thornhill, A. J.; Walshe, N. D. A.; Zanetti, C. Synthesis of the Left Hand Unit of Batzelladine F; Revision of the Reported Relative Stereochemistry. *Tetrahedron* **1999**, *55* (21), 6547−6554.
(14) Snider, B. B.; Chen, J. Synthesis of Batzelladine E and Its E Isomer. *Tetrahedron Lett.* **1998**, *39* (32), 5697−5700.
(15) Snider, B. B.; Chen, J.; Patil, A. D.; Freyer, A. J. Synthesis of the Tricyclic Portions of Batzelladines A, B and D. Revision of the Stereochemistry of Batzelladines A and D. *Tetrahedron Lett.* **1996**, *37* (39), 6977−6980.
(16) Cohen, F.; Overman, L. E. Enantioselective Total Synthesis of Batzelladine F and Definition of Its Structure. *J. Am. Chem. Soc.* **2006**, *128* (8), 2604−2608.
(17) Parr, B. T.; Economou, C.; Herzon, S. B. A Concise Synthesis of (+)-Batzelladine B from Simple Pyrrole-Based Starting Materials. *Nature* **2015**, *525* (7570), 507.
(18) Economou, C.; Romaire, J. P.; Scott, T. Z.; Parr, B. T.; Herzon, S. B. A Convergent Approach to Batzelladine Alkaloids. Total Syntheses of (+)-Batzelladine E, (−)-Dehydrobatzelladine C, and (+)-Batzelladine K. *Tetrahedron* **2018**, *74* (26), 3188−3197.
(19) Cohen, F.; Overman, L. E.; Sakata, S. K. L. Asymmetric Total Synthesis of Batzelladine D. *Org. Lett.* **1999**, *1* (13), 2169−2172.
(20) Overman, L. E.; Wolfe, J. P. Synthesis of Polycyclic Guanidines by Cyclocondensation Reactions of N -Amidinyliminium Ions. *J. Org. Chem.* **2001**, *66* (9), 3167−3175.
(21) Aron, Z. D.; Overman, L. E. The Tethered Biginelli Condensation in Natural Product Synthesis. *Chem. Commun.* **2004**, *0* (3), 253−265.
(22) Shimokawa, J.; Ishiwata, T.; Shirai, K.; Koshino, H.; Tanatani, A.; Nakata, T.; Hashimoto, Y.; Nagasawa, K. Total Synthesis of (+)-Batzelladine A and (−)-Batzelladine D, and Identification of Their Target Protein. *Chem. - Eur. J.* **2005**, *11* (23), 6878−6888.
(23) Ishiwata, T.; Hino, T.; Koshino, H.; Hashimoto, Y.; Nakata, T.; Nagasawa, K. Total Synthesis of Batzelladine D. *Org. Lett.* **2002**, *4* (17), 2921−2924.
(24) Arnold, M. A.; Day, K. A.; Durón, S. G.; Gin, D. Y. Total Synthesis of (+)-Batzelladine A and (−)-Batzelladine D via [4 + 2]-Annulation of Vinyl Carbodiimides with N -Alkyl Imines. *J. Am. Chem. Soc.* **2006**, *128* (40), 13255−13260.
(25) Evans, P. A.; Qin, J.; Robinson, J. E.; Bazin, B. Enantioselective Total Synthesis of the Polycyclic Guanidine-Containing Marine Alkaloid (−)-Batzelladine D. *Angew. Chem., Int. Ed.* **2007**, *46* (39), 7417−7419.
(26) Olszewski, A.; Sato, K.; Aron, Z. D.; Cohen, F.; Harris, A.; McDougall, B. R.; Robinson, W. E.; Overman, L. E.; Weiss, G. A. Guanidine Alkaloid Analogs as Inhibitors of HIV-1 Nef Interactions with P53, Actin, and P56lck. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (39), 14079−14084.
(27) Shi, Y.; Pierce, J. G. Synthesis of the 5,6-Dihydroxymorpholin-3-One Fragment of Monanchocidin A. *Org. Lett.* **2015**, *17* (4), 968−971.
(28) Pitts, C. R.; Lectka, T. Chemical Synthesis of β-Lactams: Asymmetric Catalysis and Other Recent Advances. *Chem. Rev.* **2014**, *114* (16), 7930−7953.
(29) Brandi, A.; Cicchi, S.; Cordero, F. M. Novel Syntheses of Azetidines and Azetidinones. *Chem. Rev.* **2008**, *108* (9), 3988−4035.
(30) Hosseyni, S.; Jarrahpour, A. Recent Advances in β-Lactam Synthesis. *Org. Biomol. Chem.* **2018**, *16* (38), 6840−6852.
(31) Deketelaere, S.; Nguyen, T. V.; Stevens, C. V.; D'hooghe, M. Synthetic Approaches toward Monocyclic 3-Amino-β-Lactams. *ChemistryOpen* **2017**, *6* (3), 301−319.
(32) Rao, A. V. R.; Gurjar, M. K.; Vasudevan, J. An Enantiospecific Synthesis of the Tricyclic Guanidine Segment of the Anti-HIV Marine Alkaloid Batzelladine A. *J. Chem. Soc., Chem. Commun.* **1995**, *0* (13), 1369.
(33) Galletti, P.; Quintavalla, A.; Ventrici, C.; Giannini, G.; Cabri, W.; Penco, S.; Gallo, G.; Vincenti, S.; Giacomini, D. Azetidinones as Zinc-Binding Groups to Design Selective HDAC8 Inhibitors. *ChemMedChem* **2009**, *4* (12), 1991−2001.
(34) Nonoshita, K.; Maruoka, K.; Yamamoto, H. Conjugate Reduction of α,β-Unsaturated Ketones with Amphiphilic Reaction System. *Bull. Chem. Soc. Jpn.* **1988**, *61* (6), 2241−2243.
(35) Bouffard, F. A.; Christensen, B. G. Thienamycin Total Synthesis: Stereocontrolled Introduction of the Hydroxyethyl Side Chain. *J. Org. Chem.* **1981**, *46* (11), 2208−2212.
(36) Aoki, H.; Okuhara, M. Natural Beta-Lactam Antibiotics. *Annu. Rev. Microbiol.* **1980**, *34* (1), 159−181.
(37) Alcaide, B.; Almendros, P.; Aragoncillo, C. β-Lactams: Versatile Building Blocks for the Stereoselective Synthesis of Non-β-Lactam Products. *Chem. Rev.* **2007**, *107* (11), 4437−4492.
(38) Toshimitsu, A.; Terao, K.; Uemura, S. Intramolecular Amidoselenation of N-Alkenyl Amides: Formation of Nitrogen Heterocycles. *J. Org. Chem.* **1986**, *51* (10), 1724−1729.
(39) Huang, X.; Li, X.; Xie, X.; Harms, K.; Riedel, R.; Meggers, E. Catalytic Asymmetric Synthesis of a Nitrogen Heterocycle through Stereocontrolled Direct Photoreaction from Electronically Excited State. *Nat. Commun.* **2017**, *8* (1), 2245.
(40) Hamed, R. B.; Henry, L.; Gomez-Castellanos, J. R.; Mecinović, J.; Ducho, C.; Sorensen, J. L.; Claridge, T. D. W.; Schofield, C. J. Crotonase Catalysis Enables Flexible Production of Functionalized Prolines and Carbapenams. *J. Am. Chem. Soc.* **2012**, *134* (1), 471−479.
(41) Bodner, M. J.; Li, R.; Phelan, R. M.; Freeman, M. F.; Moshos, K. A.; Lloyd, E. P.; Townsend, C. A. Definition of the Common and Divergent Steps in Carbapenem B-Lactam Antibiotic Biosynthesis. *ChemBioChem* **2011**, *12* (14), 2159−2165.
(42) Asada, S.; Kato, M.; Asai, K.; Ineyama, T.; Nishi, S.; Izawa, K.; Shono, T. Enantioselective Synthesis of the Carbapenem Ring System from (S)-Proline. *J. Chem. Soc., Chem. Commun.* **1989**, *0* (8), 486.

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

(43) Pieczykolan, M.; Furman, B.; Chmielewski, M. Formal Synthesis of Thienamycin. *J. Antibiot.* **2017**, *70* (6), 781−787.
(44) Nasr El Dine, A.; Gree, D.; Roisnel, T.; Caytan, E.; Hachem, A.; Gree, R. Synthesis of Fluorine-Containing Exoalkylidene B-Lactams. *Eur. J. Org. Chem.* **2016**, *2016* (3), 556−561.
(45) Chemler, S. R.; Bovino, M. T. Catalytic Aminohalogenation of Alkenes and Alkynes. *ACS Catal.* **2013**, *3* (6), 1076−1091.
(46) Faulkner, A.; Bower, J. F. Highly Efficient Narasaka-Heck Cyclizations Mediated by P(3,5-(CF3)2C6H3)3: Facile Access to N-Heterobicyclic Scaffolds. *Angew. Chem., Int. Ed.* **2012**, *51* (7), 1675−1679.
(47) Race, N. J.; Hazelden, I. R.; Faulkner, A.; Bower, J. F. Recent Developments in the Use of Aza-Heck Cyclizations for the Synthesis of Chiral N-Heterocycles. *Chem. Sci.* **2017**, *8* (8), 5248−5260.
(48) Shuler, S. A.; Yin, G.; Krause, S. B.; Vesper, C. M.; Watson, D. A. Synthesis of Secondary Unsaturated Lactams via an Aza-Heck Reaction. *J. Am. Chem. Soc.* **2016**, *138* (42), 13830−13833.
(49) Mai, D. N.; Wolfe, J. P. Asymmetric Palladium-Catalyzed Carboamination Reactions for the Synthesis of Enantiomerically Enriched 2-(Arylmethyl)- and 2-(Alkenylmethyl)Pyrrolidines. *J. Am. Chem. Soc.* **2010**, *132* (35), 12157−12159.
(50) Babij, N. R.; Wolfe, J. P. Asymmetric Total Synthesis of (+)-Merobatzelladine B. *Angew. Chem., Int. Ed.* **2012**, *51* (17), 4128−4130.
(51) Bertrand, M. B.; Neukom, J. D.; Wolfe, J. P. Mild Conditions for Pd-Catalyzed Carboamination of N -Protected Hex-4-Enylamines and 1-, 3-, and 4-Substituted Pent-4-Enylamines. Scope, Limitations, and Mechanism of Pyrrolidine Formation. *J. Org. Chem.* **2008**, *73* (22), 8851−8860.
(52) Malkov, A. V.; Barłóg, M.; Miller-Potucká, L.; Kabeshov, M. A.; Farrugia, L. J.; Kočovský, P. Stereoselective Palladium-Catalyzed Functionalization of Homoallylic Alcohols: A Convenient Synthesis of Di- and Trisubstituted Isoxazolidines and B-Amino-*δ*-Hydroxy Esters. *Chem. - Eur. J.* **2012**, *18* (22), 6873−6884.
(53) Kočovský, P.; Bäckvall, J. The Syn/Anti-Dichotomy in the Palladium-Catalyzed Addition of Nucleophiles to Alkenes. *Chem. - Eur. J.* **2015**, *21* (1), 36−56.
(54) Manzoni, M. R.; Zabawa, T. P.; Kasi, D.; Chemler, S. R. Palladium(II)-Catalyzed Intramolecular Aminobromination and Aminochlorination of Olefins. *Organometallics* **2004**, *23* (23), 5618−5621.
(55) Louwrier, S.; Tuynman, A.; Hiemstra, H. Synthesis of Bicyclic Guanidines from Pyrrolidin-2-One. *Tetrahedron* **1996**, *52* (7), 2629−2646.
(56) Barrett, A. G. M.; Graboski, G. G.; Sabat, M.; Taylor, S. J. Beta-Lactam Annulation Using (Phenylthio)Nitromethane. *J. Org. Chem.* **1987**, *52* (21), 4693−4702.
(57) Fujimoto, K.; Iwano, Y.; Hirai, K. From Penicillin to Penem and Carbapenem. IX. C 1 -Unit Introduction and the Carbapenam Synthesis from the Penicillin Molecule. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1887−1896.
(58) Garud, D. R.; Ando, H.; Kawai, Y.; Ishihara, H.; Koketsu, M. Synthesis of Novel Selenapenams, Selenacephems, and Selenazepines Using a 2-(Trimethylsilyl)Ethyl Protection Approach. *Org. Lett.* **2007**, *9* (22), 4455−4458.
(59) Garud, D. R.; Makimura, M.; Koketsu, M. Synthetic Approaches to Selenacephams and Selenacephems via a Cleavage of Diselenide and Selenium Anion. *New J. Chem.* **2011**, *35* (3), 581−586.
(60) Chou, S.-S. P.; Huang, J.-L. Tandem Cross Metathesis and Intramolecular Aza-Michael Reaction to Synthesize Bicyclic Piperidines and Indolizidine 167E. *Tetrahedron Lett.* **2012**, *53* (41), 5552−5554.
(61) Vedrenne, E.; Dupont, H.; Oualef, S.; Elkaïm, L.; Grimaud, L. Dramatic Effect of Boron-Based Lewis Acids in Cross-Metathesis Reactions. *Synlett* **2005**, No. 4, 670−672.
(62) Kim, K. S.; Qian, L. Improved Method for the Preparation of Guanidines. *Tetrahedron Lett.* **1993**, *34* (48), 7677−7680.
(63) Compound was purchased from Ambeed, Inc. 200 g/$221, CAS: 76855−69−1.
(64) Mulani, M. S.; Kamble, E. E.; Kumkar, S. N.; Tawre, M. S.; Pardesi, K. R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front. Microbiol.* **2019**, *10*, 539.
(65) Tommasi, R.; Brown, D. G.; Walkup, G. K.; Manchester, J. I.; Miller, A. A. ESKAPEing the Labyrinth of Antibacterial Discovery. *Nat. Rev. Drug Discovery* **2015**, *14* (8), 529−542.
(66) Santajit, S.; Indrawattana, N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed Res. Int.* **2016**, *2016*, 2475067.
(67) Bassetti, M.; Merelli, M.; Temperoni, C.; Astilean, A. New Antibiotics for Bad Bugs: Where Are We? *Ann. Clin. Microbiol. Antimicrob.* **2013**, *12* (1), 22.

https://dx.doi.org/10.1021/jacs.0c04091
J. Am. Chem. Soc. XXXX, XXX, XXX−XXX