Concise Synthesis and Antimicrobial Evaluation of the Guanidinium Alkaloid Batzelladine D

You-Chen Lin[a,b], Aubert Riboucourt[a], Yasamin Moazami[a†] and Joshua G. Pierce*[a,b]

[a] Department of Chemistry, College of Sciences, NC State University, 2620 Yarbrough Drive, Raleigh, NC, USA [b] Comparative Medicine Institute, NC State University, Raleigh, NC, USA

ABSTRACT: A concise synthesis of the tricyclic guanidinium alkaloid batzelladine D has been accomplished in a sequence of 8 steps from readily available building blocks. Highlights of the synthesis include gram-scale preparation of a late stage intermediate, pinpoint stereocontrol around the tricyclic skeleton and a modular strategy that enables analog generation. A key bicyclic β-lactam intermediate serves to not only control stereochemistry, but also serves as a pre-activated coupling partner to install the ester sidechain. The stereo-controlled synthesis allowed for the investigation of the antimicrobial activity of batzelladine D, demonstrating promising activity that is more potent for non-natural stereoisomers.

The marine environment is one of the most prolific sources of chemically complex and biologically active molecular scaffolds, such as polycyclic guanidinium alkaloids (PGA).[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 are known for most classes of PGAs. Efficient and modular synthetic approaches to these molecular scaffolds not only allows for comprehensive biological studies it also allows 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. 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*- stereoconfiguraiton anchored to the various side chains thorough an ester linkage. The structural complexity of these scaffolds, in addition to anti-viral and cytotoxicity activity reported by the

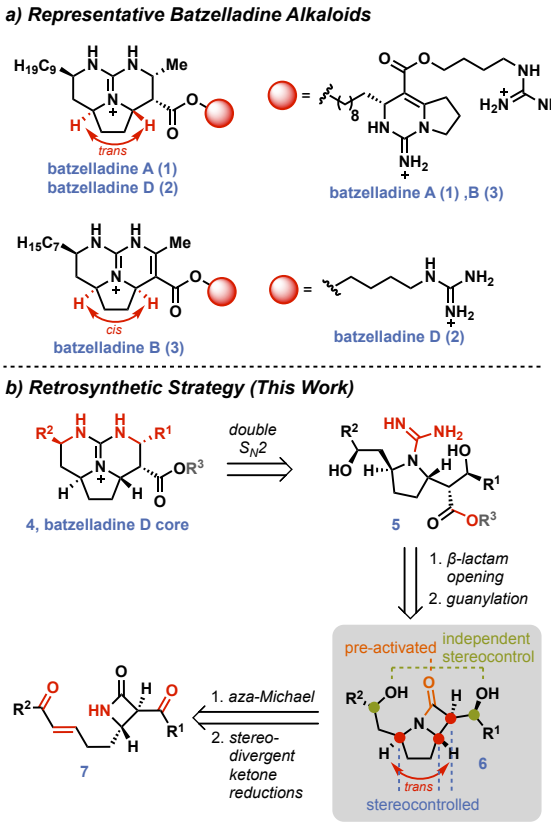

Figure 1. (a) Representative batzelladine alkaloids; (b) Retrosynthetic strategy presented herein.

isolation team, has led to significant interest from the synthetic community. C*is*-pyrrolidine members of the batzelladine family, represented by batzelladine B (**3**), were first to draw the attention of the synthetic community, with **3** itself succumbing to an elegant approach from Herzon and co-workers in 2015.[12–18] 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 sub-family was batzelladine D (**2**) by Overman and co-workers though 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

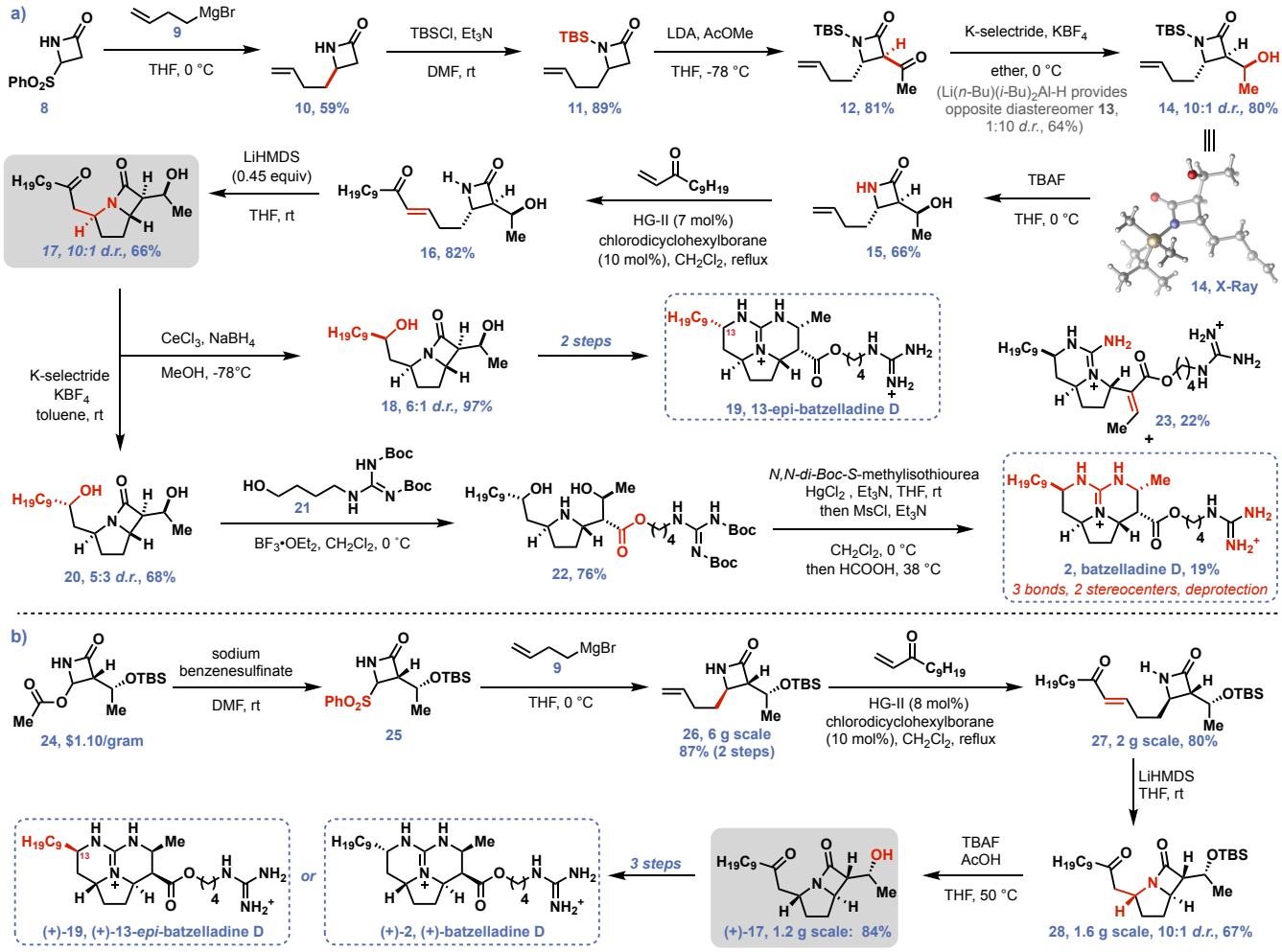

Figure 2. (a) Synthesis of (±)-batzelladine D and (±)-13-*epi*-batzelladine D. (b) Gram-scale synthesis of **29** and synthesis of (+)-batzelladine D and (+)-13-*epi*-batzelladine D

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.[4,5,7,8,10,11,22,26] 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**) did not display measurable activity. 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 sidechains such as **2**.[26] 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 explore the broader biological activities of these PGAs.

As part of a broader program targeting modular and practical syntheses of PGAs,[4,27] we became interested in developing a synthesis of batzelladine D (**2**) that would facilitate access to an array of stereochemical 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 is inspired by previous efforts but represents a 1-pot solution that should allow for the generation of all possible stereoisomers from a common intermediate. Access to the fully functionalized pyrrolidine relies on the use of a key bicyclic β-lactam intermediate (**6**, Figure 1b) that would serve not only as a pre-activated coupling partner though the lactam itself, but also serve to control the stereochemistry of the pyrrolidine core. **6** would be prepared through an aza-Michael addition of mono-cyclic 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.[28–32]

The synthesis of (±)-batzelladine D (**2**) begins with commercially available β-lactam **8** which is substituted with butenyl Grignard reagent **9**, *N*-protected with TBS and subsequently acylated to provide **12** as a single diastereomer in good yield over 3 steps (Figure 2a).[33] At this stage reduction of the methyl ketone was required, and we sought to develop conditions to access both diastereomers, ultimately enabling both natural product epimers via subsequent displacement (Figure 1b). After extensive screening (see supporting information **Table S1**) we found that Li(*n*-Bu)(*i*-Bu)$_2$Al-H provided the opposite diastereomer **13** required for batzelladine D in 10:1 *d.r.* and 64% yield while K-selectride/KBF_4 in ether provided the desired diastereomer **14** in 10:1 *d.r.* and 80% yield and the structure was confirmed through X-ray analysis.[34] Deprotection of the TBS group with TBAF provides **15** which is subjected to cross-metathesis with Hoveyda-Grubbs second generation catalyst to provide **16** in 82% yield.[35] With **16** in hand we began exploring conditions to allow for stereoselective aza-Michael addition of the β-lactam to the enone to generate the key bicyclic intermediate **17**.[36] Surprisingly, a number of basic and acidic conditions provided only starting material or complex mixtures of products, partly due to the highly reversible nature of this addition and the relative activation of the β-lactam carbonyl upon cyclization (see supporting information **Table S2**). Our best success was achieved with sub-stoichiometric LiHMDS, ultimately revealing 0.45 equivalents as the optimal conditions to provide **17** in 10:1 *d.r.* and 66% isolated yield of the major diastereomer.

With the bicyclic β-lactam **17** in hand we were now positioned to explore the selective reduction of the remaining ketone, again targeted an approach to both diastereomers while keeping the β-lactam carbonyl intact. The order of these steps is critical, as attempted opening of the β-lactam before ketone reduction results in loss of stereochemical integrity due to facile retro-aza-Michael reactions.[37] Reduction of **17** under Luche conditions selectively provided **18** in 97% yield and 6:1 *d.r.*, the opposite diastereomer required for batzelladine D, but enables access to (±)-13-*epi*-batzelladine D (**19**) via our synthetic strategy. Continued evaluation of reduction conditions (see supplementary information **Table S3**) identified K-selectride/KBF_4 in toluene as the optimal condition, generating **20** in 68% yield and 5:3 *d.r.* Subsequent opening of the β-lactam with side chain **21** proceed smoothly upon activation with BF_3•OEt to provide the target dihydroxy pyrrolidine **22**. Installation of the guanidine by treatment with *N,N*-di-Boc-*S*-methylisothiourea and mercury chloride[23,38] 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.[19] This process installed the three critical bonds of the core in a stereoselective fashion and provided (±)-batzelladine D (**2**) in 19% yield along with 22% of **23**, resulting from β-mesylate elimination under the reaction conditions. Utilizing this endgame, (±)-15-*epi*-batzelladine D (**S1**) and (±)-13-*epi*-batzelladine D (**19**), were also prepared from the corresponding alcohol epimers **13** and **18** (see SI for details).

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 while also generating gram-scale quantities of our key intermediates (Figure 2b). To access the non-natural enantiomer, we chose to start from commercially available β-lactam **24**, already bearing the necessary hydroxyethyl side chain, and available for $1.10/gram due to its use in antibiotic synthesis. **24** could be readily converted to the requisite sulfone **25** through treatment with sodium benzenesulfinate and subsequently displaced by butenyl Gringnard reagent **9** to generate **26** on 6-gram scale. Cross-methathesis, aza-Michael addition and TBS deprotection proceed smoothly as before to provide bicyclic β-lactam **20** on gram-scale, set to undergo diastereoselective reduction and conversion to (+)-batzelladine D (**2**) and (+)-13-*epi*-batzelladine D (**19**).

Table 1. Antimicrobial activity of select compounds.

	MIC values (μg/mL)					
Strain / Compound	(±)-**2**[a]	(+)-**2**[b]	(±)-**19**[c]	(+)-**19**[d]	(±)-**S1**[e]	Lin[f]
S. aureus 33591 (MRSA)	8	8	8	2	8	1
S. aureus 29213 (MSSA)	8	8	8	2	8	2
A. baumannii 19606	128	64	256	64	256	64

[a] racemic batzelladine D; [b] enantiopure batzelladine D (non-natural) [c] racemic 13-*epi*-batzelladine D; [d] enantiopure 13-*epi*-batzelladine D (non-natural); [e] racemic 15-*epi*-batzelladine D [f] Lin = linezolid (control).

As an initial exploration into the biological activity of batzelladine D and key diastereomeric and enantiomeric analogs we sought to explore their antimicrobial activity against a series of ESKAPE pathogens (Table 1).[39–41] In initial screening, (±)-batzelladine D (**2**) proved 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. It also displayed modest activity against the gram-negative pathogen *A. baumannii* with a MIC of 128 μg/mL. Upon systematic evaluation of the stereoisomers, it was revealed that non-natural stereoisomers were more active than the natural product. This effect can be highlighted by comparison of (±)-13-*epi*-batzelladine D (**19**) and (+)-13-*epi*-batzelladine D (**19**), wherein the unnatural

enantiomer is significantly more active (Table 1). The effect of stereochemistry on antimicrobial activity suggests there may be distinct targets or pathways involved in the observed activity and warrants additional study.

The synthesis of batzelladine D (**2**) and a series of stereoisomers was accomplished in 8-10 steps with key intermediates prepared on gram-scale. These efforts have provided a platform to access an array of *trans*-pyrrolidine PGAs that bear pinpoint modifications around the skeleton. The present work has resulted in identification of batzelladine D (**2**) as possessing promising levels of antimicrobial activity, with non-natural isomers being more active, particularly against gram-positive *S. aureus* strains. 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. The synthesis of additional PGAs using the underlying strategy presented herein, along with their utilization as chemical probes to study their mechanisms of action are underway and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI:

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

Crystallographic data for # (CIF)

AUTHOR INFORMATION

Corresponding Author

*jgpierce@ncsu.edu

Present Addresses

†Gilead Sciences, Seattle, Washington.

Notes

The authors declare no competing financial interest.

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SYNOPSIS TOC