Concise Synthesis and Antimicrobial Evaluation of the Guanidinium Alkaloid Batzelladine D

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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 b-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.

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Concise Synthesis and Antimicrobial Evaluation of the Guanidinium Alkaloid Batzelladine D

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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 *Batella* 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*- stereoconfiguration 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. *Cis*-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

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)₂Al-H provided the opposite diastereomer **13** required for batzelladine D in 10:1 *d.r.* and 64% yield while K-selectride/KBF₄ 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/KBF₄ 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 BF₃•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 Grignard reagent **9** to generate **26** on 6-gram scale. Cross-metathesis, 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.

Strain / Compound	MIC values (μg/mL)					
	(±)- 2 ^a	(+)- 2 ^b	(±)- 19 ^c	(+)- 19 ^d	(±)- S1 ^e	Lin ^f
<i>S. aureus</i> 33591 (MRSA)	8	8	8	2	8	1
<i>S. aureus</i> 29213 (MSSA)	8	8	8	2	8	2
<i>A. baumannii</i> 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 ¹H and ¹³C NMR spectra (PDF)

Crystallographic data for **2** (CIF)

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Notes

The authors declare no competing financial interest.

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Concise Synthesis of Polycyclic Guanidinium Alkaloid Batzelladine D

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Supporting Information

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General experimental:

General experimental procedures: All reactions were carried out under an inert argon atmosphere with anhydrous solvents under anhydrous conditions unless otherwise stated. All reactions, except those conducted in the presence of water were carried out in flame-dried apparatus. Yields refer to chromatographically yield, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) analysis (pre-coated silica gel 60 F254 plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with KMnO₄ solution with heating or iodine. Reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column). Flash chromatography on SiO₂ was used to purify the crude reaction mixtures and performed on a Biotage Isolera utilizing Biotage cartridges and linear gradients. Atom numbering of the intermediates were used as the corresponding atom number that found in the final product batzelladine D. Diastereomeric ratio was determined by crude NMR spectroscopy.

Materials: Tetrahydrofuran (THF) and dichloromethane (CH₂Cl₂) were obtained by passing the previously degassed solvents through activated alumina columns under nitrogen atmosphere. Triethylamine and *N,N*-diisopropylethylamine were distilled over CaH₂. Deuterated solvents (containing 0.03 to 0.05 vol % tetramethylsilane, TMS) were purchased from Cambridge Isotope Laboratories. All other reagents were purchased from commercial chemical companies and used without further purification, unless otherwise stated.

Instrumentation: Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus. Infrared spectra were determined on the Agilent Cary 630 FTIR spectrometer and data are represented as frequency of absorption (cm⁻¹). Optical rotation was measured on Jasco P-2000 polarimeter. ¹H and ¹³C NMR spectra were obtained on a 400, 600 or 700 MHz instrument in CDCl₃ or CD₃OD unless otherwise noted. COSY, HSQC, and where necessary NOESY and HMBC spectra were used to aid structure assignments. Chemical shifts (δ) were reported in parts per million (ppm) with the residual solvent peak used as an internal standard (CDCl₃ ¹H NMR = 7.26 ppm, ¹³C NMR = 77.16 ppm; CD₃OD ¹H NMR = 3.31, 4.87 ppm, ¹³C NMR = 49.0 ppm; TMS ¹H NMR = 0 ppm, ¹³C NMR = 0 ppm) and multiplicities are reported as observed. The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad. Low resolution mass spectra were obtained using electrospray ionization (ESI). High-resolution mass spectra were obtained on a high-resolution mass spectrometer – the Thermo Fisher Scientific Exactive Plus MS, a benchtop full-scan Orbitrap™ mass spectrometer – using Heated Electrospray Ionization (HESI). The NC state small molecule X-ray facility collected and analyzed all X-ray diffraction data.

Synthetic procedures and spectra:

β -lactam 10: Product **10** was prepared by modifying a known procedure.¹ 3-Butenyl-magnesium bromide **9**, freshly prepared from Mg (0.270 g, 11.1 mmol, 3.13 equiv), 3-butenyl bromide (1.51 g, 10.8 mmol, 3.06 equiv) and 1,2-dibromoethane (0.068 g, 0.354 mmol, 0.1 equiv) in THF (5 mL) at 25 °C, was added dropwise to a solution of 4-(phenylsulfonyl)-2-azetidinone **8** (0.770 g, 3.54 mmol) in THF (25 mL) at -78 °C. The resulting solution was warmed to rt, stirred for 12 h, quenched (sat. aq. NH₄ Cl, 50 mL), the aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic layers were combined, washed with brine (1 x 100 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 20% to 100%, EtOAc in hexanes) to provide the β -lactam **10** (0.261 g, 59%) as a colorless oil.

R_f = 0.25 (50% EtOAc in hexanes)

¹H NMR (400 MHz, CDCl₃) δ 6.14 (s, 1H), 5.79 (ddt, J = 16.9, 10.2, 6.9 Hz, 1H), 5.08–4.98 (m, 2H), 3.65–3.60 (m, 1H), 3.06 (ddd, J = 14.8, 5.0, 2.2 Hz, 1H), 2.58 (ddd, J = 14.8, 2.4, 1.3 Hz, 1H), 2.14–2.08 (m, 2H), 1.76–1.70 (m, 2H).

¹³C NMR (176 MHz, CDCl₃) δ 168.6, 137.5, 115.9, 48.2, 43.8, 34.8, 30.9.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3235, 2997, 1718, 1640, 1435, 1368, 1241, 1211, 909.

HRMS (m/z): calculated for C₇H₁₂NO [M+H]⁺ 126.0913, found 126.0912.

The characterization data are consistent with those reported in the literature¹.

— 7.26 CDCl₃

— 6.14
— 5.84
— 5.80
— 5.78
— 5.76
— 5.74
— 5.08
— 5.07
— 5.02
— 4.99
— 4.98

— 3.65
— 3.64
— 3.61
— 3.08
— 3.07
— 3.04
— 3.03
— 2.60
— 2.56
— 2.55
— 2.14
— 2.12
— 2.09
— 1.77
— 1.75
— 1.72
— 1.71
— 1.70

Solvent CDCl₃

MHz 400

Nucleus ¹H

— 168.63

— 137.49

— 115.87

— 77.16 CDCl₃

— 48.16

— 43.75

— 34.76

— 30.92

Solvent CDCl₃

MHz 176

Nucleus ¹³C

***N*-silylated β -lactam **11**:** To a solution of β -lactam **10** (141 mg, 1.13 mmol, 1.0 equiv) in DMF (1.1 mL), was added Et₃N (127 mg, 1.24 mmol, 1.1 equiv) followed by TBSCl (184 mg, 1.18 mmol, 1.1 equiv) at 25 °C. After 17 h, the reaction was quenched (sat. aq. NH₄Cl, 5mL), the aqueous layer was extracted with CH₂Cl₂ (2 x 5 mL) and the organic layers were combined, washed with H₂O (1 x 10 mL), brine (1 x 10 mL), 5% aq. LiCl (1 x 20 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 0% to 30% EtOAc in hexanes) to afford the the *N*-silylated β -lactam **11** (238 mg, 88%) as a colorless oil.

R_f = 0.45 (20% EtOAc in hexanes)

¹H NMR (700 MHz, CDCl₃) δ 5.78 (ddt, J = 16.9, 10.3, 6.6 Hz, 1H), 5.04–4.98 (m, 2H), 3.52 (ddt, J = 10.9, 5.7, 3.0 Hz, 1H), 3.10 (dd, J = 15.3, 5.4 Hz, 1H), 2.59 (dd, J = 15.3, 2.8 Hz, 1H), 2.12–1.92 (m, 3H), 1.52–1.46 (m, 1H), 0.95 (s, 9H), 0.22 (s, 3H), 0.21 (s, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 172.7, 137.3, 115.5, 49.2, 43.9, 35.4, 30.0, 26.4 (3C), 18.4, -5.2, -5.6.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\max}$: 2926, 2792, 1737, 1262, 1186, 824.

HRMS (m/z): calculated for C₁₃H₂₆NOSi [M+H]⁺ 240.1778, found 240.1777.

Ketone 12: To a solution of *N*-silylated β -lactam **11** (0.70 g, 2.92 mmol, 1.0 equiv) in THF (30 mL) at -78 °C was added LDA (17.5 mL, 17.5 mmol, 1 M in THF, 6.0 equiv). After 2 h, the methyl acetate (2.19 g, 29.2 mmol, 10 equiv) was added. The reaction was stirred at -78 °C for 30 min and then quenched (sat. aq. NH_4Cl , 50 mL), the aqueous layer was extracted with EtOAc (3 x 30 mL) and the organic layers were combined, washed with brine (1 x 50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 0% to 30% EtOAc in hexanes) to afford the ketone **12** (0.70 g, 85%) as a yellow oil.

R_f = 0.26 (10% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 5.79–5.73 (m, 1H), 5.0–4.96 (m, 2H), 3.93 (dt, J = 10.7, 3.1 Hz, 1H), 3.84 (d, J = 2.8 Hz, 1H), 2.27 (s, 3H), 2.08–2.00 (m, 2H), 1.96–1.91 (m, 1H), 1.54–1.49 (m, 1H), 0.93 (s, 9H), 0.23 (s, 3H), 0.20 (s, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 200.5, 168.0, 137.1, 116.1, 70.1, 51.3, 34.4, 30.2, 30.0, 26.4 (3C), 18.6, -5.1, -5.5.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 2930, 2792, 1741, 1707, 1167, 809.

HRMS (m/z): calculated for $\text{C}_{15}\text{H}_{27}\text{NO}_2\text{SiNa}$ $[\text{M}+\text{Na}]^+$ 304.1703, found 304.1704.

Reduction conditions of **12** (Table S1):

entry ^[a]	reductant	temperature/time	results
1	NaBH ₄	0 °C / 2 h	36% with 1:1 dr
2	Zn(BH ₄) ₂ (1 equiv)	0 °C / 1 h	35% with 2:5 dr
3	Li(<i>t</i> -BuO) ₃ Al-H	-78 °C / 0.5 h	40% with 2:5 dr
4	Li(Ph)(<i>i</i> -Bu) ₂ Al-H	-78 °C / 0.5 h	24% with 1:3 dr
5	Li(<i>sec</i> -Bu)(<i>i</i> -Bu) ₂ Al-H	-78 °C / 0.5 h	25% with 1:4 dr
6	Li(<i>n</i> -Bu)(<i>i</i> -Bu) ₂ Al-H	-78 °C / 0.5 h	56% with 1:10 dr
7	L-selectride	-78 °C / 0.5 h	40% with 1:5 dr
8	Li(<i>t</i> -BuO)(<i>i</i> -Bu) ₂ Al-H	-78 °C / 0.5 h	40% with 2:1 dr
9	DIBAL	-78 °C / 0.5 h	30% with 3:1 dr
10 ^[b]	K-selectride + KI	25 °C / 0.5 h	55% with 5:1 dr
11 ^[b]	K-selectride + KBF ₄	0 °C / 0.5 h	80% with 10:1 dr

^[a] All reactions were performed at 0.1 M in THF and 1.2 equiv reductant were used, unless otherwise noted

^[b] Reactions were performed in Et₂O

C-15-*epi*-hydroxyl β -lactam **13** : To a solution of DIBAL (1.35 mL, 1.35 mmol, 1.0 M in THF, 2.0 equiv) in THF (2 mL) at $-78\text{ }^\circ\text{C}$ was added *n*-BuLi (0.738 mL, 1.33 mmol, 1.8 M in hexanes, 2.0 equiv) dropwise. After 15 min, this solution was then added to a solution of β -lactam **12** (187 mg, 0.664 mmol, 1.0 equiv) in THF (5 mL) at $-78\text{ }^\circ\text{C}$. The reaction was stirred at $-78\text{ }^\circ\text{C}$ for 30 min and then quenched (sat. aq. NH_4Cl , 10 mL), the aqueous layer was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, washed with brine (1 x 20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 5% to 60% EtOAc in hexanes) to afford the C-15-*epi*-hydroxyl β -lactam **13** (120 mg, 64%) as a colorless oil in a 10:1 diastereomeric ratio (determined by integration of C(15)H signal).

R_f = 0.31 (30% EtOAc in hexanes)

^1H NMR (600MHz, CDCl_3) δ 5.80 (ddt, J = 16.9, 10.3, 6.5 Hz, 1H), 5.07–4.99 (m, 2H), 4.04–3.99 (m, 1H), 3.38 (dt, J = 10.5, 2.9 Hz, 1H), 2.79 (dd, J = 7.0, 2.4 Hz, 1H), 2.15–1.93 (m, 3H), 1.57–1.52 (m, 1H), 1.30 (dd, J = 6.3, 2.0 Hz, 3H), 0.96 (s, 9H), 0.25 (s, 3H), 0.22 (s, 3H).

MS (ESI^+): m/z 284 ($[\text{M}+\text{H}]^+$).

Hydroxyl β -lactam 14: To a solution of keto β -lactam **12** (110 mg, 0.390 mmol, 1.0 equiv) in Et₂O (5 mL) was added potassium tetrafluoroborate (61.5 mg, 10.8 mmol, 1.2 equiv) at 25 °C and cool to 0 °C. After 15 min, K-Selectride (0.782 mL, 0.782 mmol, 1.0 M in THF, 2.0 equiv) was added dropwise. The reaction was stirred at 0 °C for 30 min and then quenched (sat. aq. NH₄Cl, 5 mL), the aqueous layer was extracted with Et₂O (3 x 10 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 5% to 60% EtOAc in hexanes) to afford the hydroxyl β -lactam **14** (89.0 mg, 80%) as a colorless solid in a 10:1 diastereomeric ratio (determined by integration of C(15)H signal). Crystallization method: a portion of the product (2.00 mg) was dissolved in a mixture of a drop of methanol and 0.1 ml of Et₂O and crystallized by slow evaporation over 30 hours at 25 °C. Note: The yields typically ranged from 60-85% and prolonged the reaction time does not increase the yield.

R_f = 0.31 (30% EtOAc in hexanes)

¹H NMR (700 MHz, CDCl₃) δ 5.81 (ddt, J = 16.9, 10.3, 6.5 Hz, 1H), 5.07–4.97 (m, 2H), 4.13–4.08 (m, 1H), 3.53 (dt, J = 10.5, 2.9 Hz, 1H), 2.81 (dd, J = 5.8, 2.4 Hz, 1H), 2.15–2.10 (m, 1H), 2.07–2.02 (m, 1H), 1.95–1.90 (m, 1H), 1.56–1.50 (m, 1H), 1.28 (d, J = 6.6 Hz, 3H), 0.95 (s, 9H), 0.24 (s, 3H), 0.20 (s, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 174.0, 137.8, 115.6, 65.8, 64.3, 53.0, 35.0, 30.2, 26.5 (3C), 21.8, 18.6, -4.9, -5.4.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3422, 2926, 2788, 1711, 1319, 1252, 824.

HRMS (m/z): calculated for C₁₅H₃₀NO₂Si [M+H]⁺ 284.2040, found 284.2038.

A specimen of $C_{15}H_{29}NO_2Si$, approximate dimensions 0.050 mm x 0.203 mm x 0.251 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured on a Bruker-Nonius X8 Kappa APEX II system equipped with a fine-focus sealed tube ($MoK\alpha$, $\lambda = 0.71073 \text{ \AA}$) and a graphite monochromator.

The total exposure time was 36.73 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 28147 reflections to a maximum θ angle of 27.12° ($0.78 \text{ \AA}$ resolution), of which 3841 were independent (average redundancy 7.328, completeness = 99.9%, $R_{int} = 3.01\%$, $R_{sig} = 2.01\%$) and 3214 (83.68%) were greater than $2\sigma(F^2)$. The final cell constants of $a = 12.8389(5) \text{ \AA}$, $b = 11.5893(4) \text{ \AA}$, $c = 11.6900(4) \text{ \AA}$, $\beta = 90.6262(12)^\circ$, volume = $1739.30(11) \text{ \AA}^3$, are based upon the refinement of the XYZ-centroids of 9105 reflections above $20 \sigma(I)$ with $4.735^\circ < 2\theta < 54.19^\circ$. Data were corrected for absorption effects using the Multi-Scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.963. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9670 and 0.9930.

The final anisotropic full-matrix least-squares refinement on F^2 with 181 variables converged at $R1 = 3.39\%$, for the observed data and $wR2 = 9.01\%$ for all data. The goodness-of-fit was 1.029. The largest peak in the final difference electron density synthesis was $0.396 \text{ e}/\text{\AA}^3$ and the largest hole was $-0.207 \text{ e}/\text{\AA}^3$ with an RMS deviation of $0.039 \text{ e}/\text{\AA}^3$. On the basis of the final model, the calculated density was $1.083 \text{ g}/\text{cm}^3$ and $F(000)$, 624 e^- .

Sample and crystal data for 14.

Identification code	rds852
Chemical formula	C ₁₅ H ₂₉ NO ₂ Si
Formula weight	283.48 g/mol
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal size	0.050 x 0.203 x 0.251 mm
Crystal system	monoclinic
Space group	P 1 21/c 1
Unit cell dimensions	a = 12.8389(5) Å α = 90° b = 11.5893(4) Å β = 90.6262(12)° c = 11.6900(4) Å γ = 90°
Volume	1739.30(11) Å ³
Z	4
Density (calculated)	1.083 g/cm ³
Absorption coefficient	0.135 mm ⁻¹
F(000)	624
Diffractometer	Bruker-Nonius X8 Kappa APEX II
Radiation source	fine-focus sealed tube (MoKα, λ = 0.71073 Å)
Theta range for data collection	2.37 to 27.12°
Index ranges	-16 ≤ h ≤ 16, -14 ≤ k ≤ 14, -14 ≤ l ≤ 14
Reflections collected	28147
Independent reflections	3841 [R(int) = 0.0301]
Coverage of independent reflections	99.9%
Absorption correction	Multi-Scan
Max. and min. transmission	0.9930 and 0.9670
Refinement method	Full-matrix least-squares on F ²
Refinement program	SHELXL-2017/1 (Sheldrick, 2017)
Function minimized	Σ w(F _o ² - F _c ²) ²
Data / restraints / parameters	3841 / 0 / 181
Goodness-of-fit on F ²	1.029
Final R indices	3214 data; I > 2σ(I) R1 = 0.0339, wR2 = 0.0843 all data R1 = 0.0450, wR2 = 0.0901
Weighting scheme	w = 1/[σ ² (F _o ²) + (0.0429P) ² + 0.6365P] where P = (F _o ² + 2F _c ²)/3
Largest diff. peak and hole	0.396 and -0.207 eÅ ⁻³
R.M.S. deviation from mean	0.039 eÅ ⁻³

β -lactam 15: To a solution of *N*-silylated β -lactam **14** (170 mg, 0.600 mmol, 1.0 equiv) in THF (12 mL) was added TBAF (0.720 mL, 0.720 mmol, 1.0 M in THF, 1.2 equiv) dropwise at 0 °C. After 1 h, the reaction was quenched (sat. aq. NH_4Cl , 10 mL), the aqueous layer was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 12% to 100% EtOAc in hexanes) to afford the β -lactam **15** (90.0 mg, 89%) as a colorless oil.

R_f = 0.28 (70% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 6.60 (br, 1H), 5.82–5.76 (m, 1H), 5.05–4.96 (m, 2H), 4.15–4.13 (m, 1H), 3.64 (td, J = 6.8, 5.7, 3.3 Hz, 1H), 3.17 (br, 1H), 2.79–2.75 (m, 1H), 2.12–2.09 (m, 2H), 1.73–1.70 (m, 2H), 1.25 (dd, J = 6.5, 2.9 Hz, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 169.6, 137.5, 115.6, 64.6, 63.8 51.1, 34.2, 30.8, 21.6.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3250, 2971, 2926, 1718, 1372, 1085

HRMS (m/z): calculated for $\text{C}_9\text{H}_{16}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 170.1176, found 170.1175.

Dodec-1-en-3-one S4: To a solution of decanal (3.60 g, 21.9 mmol, 1.0 equiv) in Et₂O (49 mL) was added vinylmagnesium bromide (43.8 mL, 43.8 mmol, 1 M solution in THF, 2.0 equiv), and the reaction was stirred at 0 °C for 1 h and then warmed to rt. After 8 h, the reaction was quenched (sat. aq. NH₄Cl, 5 mL), the aqueous layer was extracted with Et₂O (3 x 60 mL) and the organic layers were combined, washed with brine (1 x 100 mL), dried (Na₂SO₄) and concentrated under reduced pressure to afford the crude alcohol **S3** as a colorless liquid. *R*_f 0.24 (10% EtOAc in hexanes). The crude product was used in the next step without further purification.

MnO₂ (40.6 g, 420 mol, 20 equiv) was heated in a reduced pressure oven at 100 °C overnight and then added to a flask containing alcohol **S3** (4.03 g, 21.0 mmol, 1.0 equiv) in CH₂Cl₂ (210 mL). The heterogenous mixture was heated to reflux. After 48 h, the solid was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 0% to 20%, EtOAc in hexanes) to provide the enone **S4** (2.04 g, 53%) as a light yellow oil.

¹H NMR (700 MHz, CDCl₃) δ 6.34 (dd, *J* = 17.7, 10.6 Hz, 1H), 6.21 (d, *J* = 17.7 Hz, 1H), 5.80 (d, *J* = 10.7 Hz, 1H), 2.57 (t, *J* = 7.5 Hz, 2H), 1.61 (p, *J* = 7.3 Hz, 2H), 1.31–1.22 (m, 12H), 0.87 (t, *J* = 7.1 Hz, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 201.3, 136.7, 128.0, 39.8, 32.0, 29.6, 29.6, 29.4 (2C), 24.2, 22.8, 14.2.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 2922, 2851, 1700, 1681, 1399, 958.

The characterization data are consistent with reported data.²

β -lactam 16: To a solution of β -lactam **15** (200 mg, 1.18 mmol, 1.0 equiv) and enone **S4** (609 mg, 3.31 mmol, 2.8 equiv) in degassed CH_2Cl_2 (10 mL) was added chlorodicyclohexylborane (0.120 mL, 0.120 mmol, 1.0 M in hexane, 0.1 equiv) at 25 °C. After 15 min, second-generation Hoveyda-Grubbs catalyst (65.0 mg, 0.100 mmol, 0.08 equiv) was added. The reaction was heated to reflux for 16 h and then concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 20% to 100% EtOAc in hexanes) to afford the β -lactam **16** (314 mg, 82%) as a black oil.

R_f = 0.15 (70% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 6.80 (dt, J = 15.8, 6.8 Hz, 1H), 6.46 (br, 1H), 6.14 (d, J = 15.8 Hz, 1H), 4.17–4.14 (m, 1H), 3.67 (td, J = 6.7, 2.1 Hz, 1H), 2.80 (dd, J = 5.7, 2.1 Hz, 1H), 2.68 (br, 1H), 2.50 (t, J = 7.5 Hz, 2H), 2.31–2.27 (m, 2H), 1.86–1.77 (m, 2H), 1.60–1.55 (m, 2H), 1.29–1.22 (m, 15H), 0.86 (t, J = 6.9 Hz, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 200.7, 168.8, 144.9, 130.7, 64.8, 63.8, 50.9, 40.5, 33.4, 31.8, 29.4, 29.4, 29.2, 29.2, 29.2, 24.1, 22.6, 21.6, 14.0.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3280, 2922, 2768, 1737, 1666, 1372.

HRMS (m/z): calculated for $\text{C}_{19}\text{H}_{34}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 324.2533, found 324.2532.

Model substrate conditions for aza-Michael cyclization (Table S2):

entry ^[a]	reagent / solvent	temperature	results
1	ZrCl ₄ (20 mol%) / CH ₂ Cl ₂	rt	no reaction
2	AuCl (10 mol%) / toluene	110 °C	no reaction
3	PtCl ₄ (10 mol%) / CH ₂ Cl ₂	rt	no reaction
4	ReCl ₅ (10 mol%) / CH ₂ Cl ₂	rt	no reaction
5	BF ₃ • Et ₂ O (20% mol) / CH ₂ Cl ₂	rt	no reaction
6	HCl (50 mol%) / Et ₂ O	rt	complex mixture
7	K- <i>t</i> -butoxide (2 equiv) / THF	-78 °C	complex mixture
8	K- <i>t</i> -butoxide (5 equiv) / THF	45 °C	trace product
9	TBAH (0.2 equiv) / THF	rt	no reaction
10	LDA (2 equiv) / THF	-78 °C	complex mixture
11	EtMgBr (2 equiv) / THF	0 °C	no reaction
12	NaH (2.2 equiv) / THF	0 °C	no reaction
13	LiHMDS (2.2 equiv)	rt	trace product
14 ^[b]	LiHMDS (1.2equiv)	rt	39 % ^[c]
15 ^[b]	LiHMDS (0.7 equiv)	rt	89% ^[c]
16 ^[b]	KHMDS (0.7 equiv)	rt	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] Single diastereoisomer was observed

Bicyclic β -lactam 17: To a solution of β -lactam **16** (118 mg, 0.365 mmol, 1.0 equiv) in THF (23 mL) at 25 °C was added LiHMDS (0.164 mL, 0.164 mmol, 1.0 M in THF, 0.45 equiv). After 15 min, the reaction was quenched (sat. aq. NH_4Cl , 5 mL), the aqueous layer was extracted with EtOAc (3 x 15 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated under reduced pressure to provide a 9:1 diastereomeric ratio mixture. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 12% to 100% EtOAc in hexanes) to afford the bicyclic β -lactam **17** as a colorless oil (78.2 mg, 66%)

R_f = 0.20 (50% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 4.18–4.13 (m, 2H), 3.68 (ddd, J = 8.0, 5.8, 1.9 Hz, 1H), 2.81 (dd, J = 6.6, 1.9 Hz, 1H), 2.69 (dd, J = 16.6, 5.8 Hz, 1H), 2.49 (dd, J = 16.6, 7.7 Hz, 1H), 2.49–2.45 (m, 1H), 2.41 (t, J = 7.5 Hz, 2H), 2.18 (dtd, J = 12.2, 6.1, 2.5 Hz, 1H), 1.88 (br, 1H), 1.76–1.72 (m, 1H), 1.56–1.51 (m, 3H), 1.30 (d, J = 6.3 Hz, 3H), 1.28–1.23 (m, 12H), 0.87 (t, J = 7.1 Hz, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 208.8, 176.3, 65.9, 63.2, 55.2, 54.2, 48.0, 43.1, 37.5, 31.8, 30.6, 29.4, 29.4, 29.2, 29.1, 23.6, 22.6, 21.8, 14.1.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3407, 3038, 2922, 2773, 1748, 1659.

HRMS (m/z): calculated for $\text{C}_{19}\text{H}_{34}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 324.2533, found 324.2530.

[In the asymmetric route, $[\alpha]_{\text{D}}^{24}$: +56.9°, (c = 0.75, CHCl_3)]

Reduction conditions of **17** (Table S3):

entry ^[a]	reductant	solvent	temperature/time	results ^[b]
1	DIBAL (1.5 equiv)	THF	-78 °C / 3 hr	No DP observed ^[c]
2	DIBAL (0.75equiv)	THF	-78 °C to rt / 15 hr	SM observed ^[c]
3	LiBH ₄ (1.1 equiv)	THF	-78 °C / 3 hr	DP observed ^[c]
4	K-selectride (1.2 equiv)	THF	-78 °C / 0.5 h	24% with 1:1 dr
5	Li(<i>t</i> -BuO) ₃ Al-H (1.2 equiv)	THF	-78 °C / 0.5 h	60% with 1:1 dr
6	Zn(BH ₄) ₂ (1 equiv)	THF	-78 °C / 0.5 h	50% with 4:5 dr
7	Zn(BH ₄) ₂ (1 equiv)	THF	0 °C to rt / 21 hr	DP to over reduce product 2:1
8	Zn(BH ₄) ₂ (1 equiv)	THF	-35 °C / 20 hr	DP to over reduce product 6:1
9	Zn(BH ₄) ₂ (1 equiv)	THF	-3 °C / 12 hr	(77%) with 3:1 dr
10	CBS (10 mol%) BH ₃ -THF (0.65 equiv)	THF	0 °C / 0.5 hr	SM observed ^[c]
11	CeCl ₃ (20 mol%) NaBH ₄ (2 equiv)	MeOH	-25 °C / 1 hr	50% 4:1 dr
12	CeCl ₃ (20 mol%) NaBH ₄ (2 equiv)	MeOH	-78 °C / 5 mins	97% 6:1 dr
13	L-selectride (1.2 equiv)	THF	-78 °C / 30 mins	45% 5:1 dr
14	K-selectride (1.2 equiv)	Et ₂ O	-78 °C / 30 mins	40% 3:4 dr
15	K-selectride (1.2 equiv)	Et ₂ O	25 °C / 3 mins	45% 2:3 dr
16	K-selectride (1.15 equiv) KBF ₄ (1.2 equiv)	0.01 M toluene	25 °C / 3 mins	68% 3:5 dr
17	K-selectride (0.97 equiv) KBF ₄ (1.2 equiv)	0.01 M Et ₂ O	25 °C / 3 mins	59% 4:5 dr
18	K-selectride (0.97 equiv) KBF ₄ (1.2 equiv)	0.01 M Et ₂ O	0 °C / 3 mins	4:5 dr
19	K-selectride (0.97 equiv) KBF ₄ (4.2 equiv)	0.01 M Et ₂ O	25 °C / 3 mins	4:5 dr
20	K-selectride (1.03 equiv) KBF ₄ (1.2 equiv)	Et ₂ O	25 °C / 3 mins	64% 4:5 dr
21	K-selectride (1.03 equiv) KBF ₄ (1.2 equiv)	Et ₂ O	25 °C / 3 mins	64% 4:5 dr
22	K-selectride (1.5 equiv) KBF ₄ (1.2 equiv)	Et ₂ O	25 °C / 3 mins	4:5 dr

[a] All reactions were performed at 0.1 M, unless otherwise noted

[b] Reactions were monitored by LCMS.

[c] Over reduce product X dominated.

C-13-*epi*-dihydroxyl-bicyclic β -lactam **18:** To a solution of bicyclic β -lactam **17** (69.4 mg, 0.215 mmol, 1.0 equiv) in MeOH (2 mL) was added cerium (III) chloride (21.1 mg, 0.086 mmol, 0.4 equiv) and cool to -78°C . After 30 min, sodium borohydride (16.4 mg, 0.429 mmol, 2.0 equiv) was added in one portion. The reaction was stirred at -78°C for 1 min and then quenched (sat. aq. NH_4Cl , 5 mL), the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 5% to 100% EtOAc in hexanes) to afford the C-13-*epi*-dihydroxyl-bicyclic β -lactam **18** (67.8 mg, 97 %) as a colorless oil in a 6:1 diastereomeric ratio (determined by integration of C(15)H signal).

$R_f = 0.22$ (70% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 4.19–4.13 (m, 1H), 3.84–3.78 (m, 1H), 3.77–3.70 (m, 2H), 2.84 (dd, $J = 6.0, 2.2$ Hz, 1H), 2.38–2.29 (m, 1H), 2.21–2.13 (m, 1H), 1.80–1.74 (m, 1H), 1.58–1.54 (m, 1H), 1.51–1.39 (m, 6H), 1.28 (d, $J = 6.0$ Hz, 3H), 1.24 (s, 12H), 0.86 (t, $J = 7.0$ Hz, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 177.8, 71.3, 65.4, 63.2, 57.7, 55.3, 41.8, 37.7, 37.3, 31.9, 30.2, 29.7, 29.7, 29.6, 29.3, 25.5, 22.7, 21.7, 14.1.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3407, 2922, 2851, 1729, 1457, 1085.

HRMS (m/z): calculated for $\text{C}_{19}\text{H}_{36}\text{NO}_3$ $[\text{M}+\text{H}]^+$ 326.2690, found 326.2687.

[In the asymmetric route, $[\alpha]_{\text{D}}^{25}$: +57.9°, ($c = 1.2$, CHCl_3)]

Dihydroxyl-bicyclic β -lactam **20:** To a solution of bicyclic β -lactam **17** (19.0 mg, 0.059 mmol, 1.0 equiv) in toluene (6 mL) was added potassium tetrafluoroborate (9.24 mg, 0.070 mmol, 1.2 equiv) and cool to 0 °C. After 15 min, K-Selectride (0.068 mL, 0.068 mmol, 1.0 M in THF, 1.15 equiv) was added dropwise. The reaction was stirred at 0 °C for 15 min and then quenched (sat. aq. NH₄Cl, 5 mL), the aqueous layer was extracted with EtOAc (3 x 5 mL) and the organic layers were combined, dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 5% to 100% EtOAc in hexanes) to afford the dihydroxyl-bicyclic β -lactam **20** (13.0 mg, 68%) as a colorless oil in a 5:3 diastereomeric ratio (determined by integration of C(13)H signal).

R_f = 0.22 (70% EtOAc in hexanes)

¹H NMR (700 MHz, CDCl₃) δ 4.2–4.17 (m, 1H), 3.97–3.92 (m, 1H), 3.79–3.74 (m, 1H), 3.69 (ddd, J = 9.0, 5.5, 1.9 Hz, 1H), 3.16 (br, 1H), 2.87 (dd, J = 6.5, 1.9 Hz, 1H), 2.35 (dtd, J = 13.1, 6.7, 1.7 Hz, 1H), 2.21 (dtd, J = 11.8, 5.9, 1.6 Hz, 1H), 1.79 (m, 1H), 1.74 (s, 1H), 1.55–1.37 (m, 7H), 1.33 (d, J = 6.2 Hz, 3H), 1.26 (m, 12H), 0.87 (d, J = 7.2 Hz, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 178.4, 69.6, 65.7, 62.8, 56.3, 55.2, 41.9, 37.6, 37.3, 31.9, 31.1, 29.8, 29.7, 29.6, 29.3, 25.9, 22.7, 21.8, 14.1.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\max}$: 3418, 2922, 2855, 1733, 1457, 1088.

HRMS (m/z): calculated for C₁₉H₃₆NO₃ [M+H]⁺ 326.2690, found 326.2698.

[In the asymmetric route, [α]_D²⁴: +25.9°, (c = 0.21, CHCl₃)]

Boc-protected guanidine **21:** According to a known procedure³ To a solution of 4-amino-1-butanol (800 mg, 8.80 mmol, 1.0 equiv) in CH₂Cl₂ (68 mL) were added Et₃N (1.24 mL, 8.80 mmol, 1.0 equiv) and *N,N*-bis-Boc-1-Guanylpazole (3.09 g, 9.76 mmol, 1.1 equiv) at 25 °C. After 20 h, the reaction was quenched (sat. aq. NH₄Cl, 40 mL), the aqueous layer was extracted with CH₂Cl₂ (3 x 40 mL) and the organic layers were combined, washed with brine (1 x 80 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 12% to 100% EtOAc in hexanes) to afford the Boc-protected guanidine **21** as a white solid (2.36 g, 81%).

R_f = 0.56 (50% EtOAc in hexanes)

¹H NMR (700 MHz, CDCl₃) δ 11.48 (br, 1H), 8.37 (br, 1H), 3.69 (t, *J* = 6.2 Hz, 2H), 3.46–3.43 (m, 2H), 1.97 (br, 1H), 1.70–1.63 (m, 4H), 1.49 (d, *J* = 4.1 Hz, 18H)

¹³C NMR (176 MHz, CDCl₃) δ 163.7, 156.4, 153.4, 83.3, 79.4, 62.3, 40.5, 29.6, 28.4 (3C), 28.2 (3C), 25.7.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3336, 3116, 2979, 2918.

MS (ESI⁺): *m/z* 332 ([M+H]⁺).

The characterization data are consistent with reported data.³

pyrrolidine 22: To a solution of 5:3 mixture of bicyclic β -lactam **20** and **18** (32.6 mg, 0.100 mmol, 1.0 equiv) and **21** (34.9 mg, 0.105 mmol, 1.1 equiv) in CH₂Cl₂ (2 mL) at 0 °C was added boron trifluoride diethyl etherate (0.029 mL, 48% BF₃ basis, 0.110 mmol, 1.1 equiv). After 30 mins, the reaction was quenched (sat. aq. NaHCO₃, 3 mL), the aqueous layer was extracted with CH₂Cl₂ (3 x 5 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The residue was filtered through SiO₂ with EtOAc to remove the excess **21** and the filter cake was washed with 20% MeOH in EtOAc. The filtrate was concentrated under reduced pressure to give the crude pyrrolidine **22** (60.0 mg, 91%) as a colorless oil, which was used next step without further purification.

R_f = 0.1 (10% MeOH in EtOAc)

MS (ESI⁺): m/z 657 ([M+H]⁺).

HRMS (m/z): calculated for C₃₄H₆₅N₄O₈ [M+H]⁺ 657.4797, found 657.4786.

C-13-*epi*-pyrrolidine S5: To a solution of bicyclic β -lactam **18** (67.8 mg, 0.208 mmol, 1.0 equiv) and **21** (72.5 mg, 0.219 mmol, 1.1 equiv) in CH₂Cl₂ (4 mL) at 0 °C was added boron trifluoride diethyl etherate (0.060 mL, 48% BF₃ basis, 0.229 mmol, 1.1 equiv). After 30 mins, the reaction was quenched (sat. aq. NaHCO₃, 3 mL), the aqueous layer was extracted with CH₂Cl₂ (3 x 5 mL) and the organic layers were combined, washed with brine (1 x 10 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The residue was filtered through SiO₂ with EtOAc to remove the excess **21** and the filter cake was washed with 20% MeOH in EtOAc. The filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 0% to 20%, MeOH in EtOAc) to provide the C-13-*epi*-pyrrolidine **S5** (81 mg, 59%) as a colorless oil.

R_f = 0.1 (10% MeOH in EtOAc)

¹H NMR (700 MHz, CDCl₃) δ 11.49 (s, 1H), 8.33 (s, 1H), 4.17–4.11 (m, 3H), 3.86–3.82 (m, 1H), 3.69–3.64 (m, 1H), 3.61–3.55 (m, 1H), 3.46–3.43 (m, 2H), 2.65 (dd, J = 8.0, 3.6 Hz, 1H), 2.12 (ddd, J = 7.2, 5.5, 2.0 Hz, 1H), 2.04–2.00 (m, 1H), 1.74–1.68 (m, 2H), 1.67–1.62 (m, 2H), 1.61–1.58 (m, 1H), 1.56–1.52 (m, 2H), 1.49 (d, J = 4.0 Hz, 18H), 1.44–1.40 (m, 2H), 1.29–1.24 (m, 18H), 0.87 (t, J = 7.0 Hz, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 173.4, 163.7, 156.3, 153.5, 83.4, 79.5, 72.3, 66.6, 64.9, 60.0, 56.8, 54.0, 40.4, 40.3, 38.3, 32.5, 32.0, 29.8, 29.8, 29.7, 29.5, 29.5, 28.5, 28.2, 26.0, 25.9, 25.8, 22.8, 21.9, 14.3.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\max}$: 3329, 2922, 2743, 1718, 1636, 1614, 1331, 1133.

MS (ESI⁺): m/z 657 ([M+H]⁺).

HRMS (m/z): calculated for C₃₄H₆₅N₄O₈ [M+H]⁺ 657.4797, found 657.4786.

Batzelladine D (2): To a solution of crude pyrrolidine **22** (38.7 mg, 0.059 mmol, 1.0 equiv) in THF (1 mL) was added 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (45.0 mg, 0.147 mmol, 2.5 equiv), triethylamine (41.5 μ L, 0.295 mmol, 5.0 equiv), and HgCl₂ (38.6 mg, 0.14 mmol, 2.4 equiv) at 25 °C. After 30 min, the reaction mixture was diluted with EtOAc (2 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, redissolved in CH₂Cl₂ (5 mL) and cooled to 0 °C then triethylamine (53.4 μ L, 0.379 mmol, 6.5 equiv) and methanesulfonyl chloride (0.127 mL, 1.63 mmol, 28 equiv) was added. After 30 min, the reaction was quenched (sat. aq. NH₄Cl, 3 mL), the aqueous layer was extracted with CH₂Cl₂ (3 x 5 mL) and the organic layers were combined, dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was redissolved in formic acid (1 mL) and heated to 38 °C. After 15 h, the reaction mixture was concentrated under reduced pressure and the crude residue was purified by HPLC (C18 Column, 5% to 38% MeCN in H₂O, 0.1% formic acid, 50 minutes ramp) to afford the batzelladine D (**2**) formate salt (5.28 mg, 19%) as a white amorphous solid.

R_f = 0.2 (20% MeOH in CH₂Cl₂)

¹H NMR (600 MHz, CDCl₃) δ 4.21 (t, J = 6.4 Hz, 2H), 4.00–3.94 (m, 1H), 3.89–3.85 (m, 1H), 3.59–3.52 (m, 2H), 3.23 (t, J = 7.0 Hz, 2H), 3.16 (dd, J = 4.8, 3.3 Hz, 1H), 2.37 (ddd, J = 12.9, 5.1, 2.4 Hz, 1H), 2.28–2.21 (m, 2H), 1.80–1.71 (m, 3H), 1.69–1.58 (m, 5H), 1.48–1.29 (m, 18H), 0.91 (t, J = 6.9 Hz, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 170.6, 158.7, 151.5, 65.4, 57.8, 57.3, 53.2, 49.9, 45.5, 42.0, 37.0, 34.2, 33.1, 31.4, 30.7, 30.6, 30.5, 29.4, 26.9, 26.6, 26.2, 23.7, 18.5, 14.4.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\max}$: 3310, 3206, 2922, 2855, 1647, 1580, 1342.

HRMS (m/z): calculated for C₂₅H₄₇N₆O₂ [M+H]⁺ 463.3755, found 463.3749.

[In the asymmetric route, $[\alpha]_D^{23}$: +8.8°, (c = 0.08, MeOH)]

Comparison of synthetic and natural batzelladine D (2):

Comparison of natural (Patil)^x and synthetic batzelladine D by ¹H NMR

	Natural (600 MHz,CD ₃ OD)	Synthetic (700 MHz,CD ₃ OD)	δ ppm
H5	4.21, t	4.21, t	—
H8	3.93, m	3.97, m	0.04
H15	3.83, m	3.87, m	0.04
H11	3.55, m	3.56, m	0.01
H13	3.54, m	3.54, m	—
H2	3.21, t	3.23, t	0.02
H7	3.12, dd	3.16, dd	0.04
H10'	2.35, m	2.37, ddd	0.02
H12'	2.35, m	2.24, m	0.11
H9'	2.24, m	2.24, m	—
H4	1.75, m	1.75, m	—
H3	1.69, m	1.69, m	—
H17'	1.64, m	1.64, m	—
H9	1.60, m	1.60, m	—
H17	1.55, m	1.58, m	0.03
H12	1.42, m	1.45, m	0.03
H10	1.41, m	1.41, m	—
H16	1.28, d	1.30, m	0.02
H18	1.29, br	1.30, m	0.01
H19	1.29, br	1.30, m	0.01
H20	1.29, br	1.30, m	0.01
H21	1.29, br	1.30, m	0.01
H22	1.29, br	1.30, m	0.01
H23	1.29, br	1.30, m	0.01
H24	1.29, br	1.30, m	0.01
H25	0.89, t	0.91, t	0.02

The chemical shift discrepancy of H12' may have been a tabulation error of the original report.

Comparison of natural (Patil)^x and synthetic batzelladine D by ¹³C NMR

	Natural (150 MHz,CD ₃ OD)	Synthetic (176 MHz,CD ₃ OD)	δ ppm
C6	170.6	170.6	—
C1	158.7	158.7	—
C14	151.5	151.5	—
C5	65.4	65.4	—
C8	57.7	57.8	0.1
C11	57.3	57.3	—
C13	53.2	53.2	—
C15	49.8	49.9	0.1
C7	45.6	45.5	0.1
C2	42.0	42.0	—
C17	37.0	37.0	—
C12	34.2	34.2	—
C23	33.0	33.1	0.1
C10	31.4	31.4	—
C19	*	30.7	*
C20	*	30.6	*
C21	*	30.5	*
C22	*	*	*
C9	29.3	29.4	0.1
C4	27.0	26.9	0.1
C3	26.6	26.6	—
C18	26.2	26.2	—
C24	23.7	23.7	—
C16	18.4	18.5	0.1
C25	14.3	14.4	0.1

* = not determined

Characterization data of analogs:

S1

C-15-*epi* analog S1 was prepared by the same procedure starting C-15-*epi*-hydroxyl β -lactam **13** to afford the C-15-*epi* formate salt **S1** (5.30 mg, 13%) as a white amorphous solid.

R_f = 0.2 (20% MeOH in CH₂Cl₂)

¹H NMR (700 MHz, CD₃OD) δ 4.69–4.65 (m, 1H), 4.19 (t, J = 6.6 Hz, 2H), 4.06–4.00 (m, 2H), 3.75–3.71 (m, 1H), 3.22 (t, J = 7 Hz, 2H), 3.14 (d, J = 3.5 Hz, 1H), 2.44 (dt, J = 13.5, 3.2 Hz, 1H), 2.33–2.29 (m, 2H), 1.91–1.86 (m, 1H), 1.82–1.64 (m, 7H), 1.35–1.29 (m, 18H), 0.91 (t, J = 6.9 Hz, 3H).

¹³C NMR (176 MHz, CD₃OD) δ 171.5, 158.7, 155.4, 85.6, 65.9, 56.5, 54.9, 50.6, 45.1, 42.0, 36.0, 34.2, 33.1, 31.7, 30.6, 30.6, 30.4, 30.4, 28.7, 26.8, 26.5, 25.9, 23.7, 23.3, 14.4.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\max}$: 3366, 3191, 2905, 2800, 1677, 1200, 1133.

HRMS (m/z): calculated for C₂₅H₄₇N₆O₂ [M+H]⁺ 463.3755, found 463.3755.

19

C-13-*epi* analog 19 was prepared by the same procedure starting C-13-*epi*-dihydroxyl -bicyclic β -lactam **18** to afford the C-13-*epi* formate salt **19** (6.00 mg, 13%) as a white amorphous solid.

.

$R_f = 0.2$ (20% MeOH in CH_2Cl_2)

^1H NMR (700 MHz, CD_3OD) ^1H NMR (700 MHz, Methanol- d_4) δ 4.20 (td, $J = 6.4, 2.9$ Hz, 2H), 3.99–3.96 (m, 1H), 3.88–3.84 (m, 1H), 3.61 (q, $J = 6.8$ Hz, 1H), 3.52–3.47 (m, 1H), 3.21 (t, $J = 7.0$ Hz, 2H), 3.15 (dd, $J = 4.7, 3.2$ Hz, 1H), 2.29–2.21 (m, 2H), 2.19–2.15 (m, 1H), 1.77–1.71 (m, 3H), 1.69–1.57 (m, 5H), 1.51–1.46 (m, 1H), 1.42–1.38 (m, 2H), 1.37–1.30 (m, 12H), 1.30 (d, $J = 6.7$ Hz, 3H), 0.91 (t, $J = 7.0$ Hz, 3H).

^{13}C NMR (176 MHz, CD_3OD) δ 170.6, 158.7, 151.4, 65.4, 58.0, 53.5, 51.2, 50.0, 49.5, 45.4, 42.0, 38.9, 33.1, 31.7, 31.6, 30.7, 30.6, 30.5, 29.4, 26.9, 26.6, 26.5, 23.7, 18.4, 14.4.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3280, 3138, 2922, 2810, 1729, 1647, 1695, 1342.

HRMS (m/z): calculated for $\text{C}_{25}\text{H}_{47}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$ 463.3755, found 463.3750.

[In the asymmetric route, $[\alpha]_{\text{D}}^{23}$: +33.2°, ($c = 0.09$, MeOH)]

C-13-elim analog S2 was prepared by the same procedure starting C-13-*epi*-dihydroxyl-bicyclic β -lactam **18** to afford the C-13-*elim* formate salt **S2** (7.2 mg, 15%) as a white amorphous solid.

R_f = 0.2 (20% MeOH in CH_2Cl_2)

^1H NMR (700 MHz, CD_3OD) δ 7.08 (q, J = 7.4 Hz, 1H), 4.96 (t, J = 8.7 Hz, 1H), 4.20 (td, J = 6.4, 2.4 Hz, 2H), 4.05–4.00 (m, 1H), 3.55–3.52 (m, 1H), 3.22 (t, J = 7.1 Hz, 2H), 2.43 (dt, J = 12.6, 7.4 Hz, 1H), 2.30 (dt, J = 11.8, 5.8 Hz, 1H), 2.26 (ddd, J = 13.4, 3.9, 1.7 Hz, 1H), 2.01–1.98 (m, 1H), 1.96 (d, J = 7.4 Hz, 3H), 1.78–1.74 (m, 2H), 1.71–1.66 (m, 2H), 1.64–1.59 (m, 2H), 1.58–1.47 (m, 2H), 1.42–1.30 (m, 14H), 0.91 (t, J = 7.0 Hz, 3H).

^{13}C NMR (176 MHz, CD_3OD) δ 166.4, 158.7, 153.1, 143.0, 132.3, 65.3, 57.7, 55.7, 50.1, 42.1, 37.1, 33.1, 33.0, 31.9, 31.2, 30.7, 30.6, 30.5, 30.5, 27.0, 26.8, 26.6, 23.7, 14.4, 14.2.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3310, 3163, 2922, 2866, 1633, 1603, 1342.

HRMS (m/z): calculated for $\text{C}_{25}\text{H}_{47}\text{N}_6\text{O}_2$ $[\text{M}+\text{H}]^+$ 463.3755, found 463.3749.

[In the asymmetric route, $[\alpha]_{\text{D}}^{24}$: +10.2°, (c = 0.12, MeOH)]

Synthesis of enantioenriched bicyclic β -lactam 17:

β -lactam 26: To a solution of (3*R*)-3-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)ethyl)-4-oxoazetidin-2-yl acetate (6.40 g, 22.3 mmol, 1.0 equiv, CAS: 76855-69-1 from Ambeed) in DMF (55 mL), was added sodium phenyl sulfinate (8.04 g, 49.0 mmol, 2.2 equiv) at 25 °C. After 20 h, the reaction was diluted with Et₂O (50 mL), quenched (H₂O, 5 mL), the aqueous layer was extracted with Et₂O (3 x 50 mL) and the organic layers were combined, washed with, H₂O (1 x 200 mL), brine (1 x 200 mL), 5% aq. LiCl (1 x 200 mL), dried (Na₂SO₄) and concentrated under reduced pressure to afford the sulfonyl β -lactam **25** as a white solid. The crude material was used in the next step without further purification.

3-Butenyl-magnesium bromide, freshly prepared from Mg (2.24 g, 92.0 mmol, 4.3 equiv), 3-butenyl bromide (12.4 g, 89.2 mmol, 4.1 equiv) and 1,2-dibromoethane (0.414 g, 2.16 mmol, 0.1 equiv) in THF (71 mL) at 25 °C, was added dropwise to a solution of the above crude sulfonyl β -lactam **25** (8.23 g, 21.6 mmol, 1.0 equiv) in THF (108 mL) at -78 °C. Then the solution was warmed to rt. After 12 h, the reaction was quenched (sat. aq. NH₄Cl, 5 mL), the aqueous layer was extracted with EtOAc (3 x 100 mL) and the organic layers were combined, washed with brine (1 x 150 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO₂, gradient elution: 0% to 60%, EtOAc in hexanes) to provide the β -lactam **26** (5.30 g, 87%) as a white solid.

R_f = 0.38 (30% EtOAc in hexanes)

¹H NMR (700 MHz, CDCl₃) δ 6.00 (s, 1H), 5.81 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H), 5.07–4.99 (m, 2H), 4.18–4.13 (m, 1H), 3.64 (ddd, J = 8.1, 6.1, 2.2 Hz, 1H), 2.74 (ddd, J = 5.3, 2.2, 1.1 Hz, 1H), 2.12 (m, 2H), 1.75–1.70 (m, 2H), 1.22 (d, J = 6.3 Hz, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H).

¹³C NMR (176 MHz, CDCl₃) δ 168.9, 137.6, 115.5, 65.8, 64.6, 51.2, 34.4, 31.1, 25.9 (3C), 22.9, 181, -4.1, -4.8.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3250, 3097, 2926, 2851, 1762, 1662, 835.

HRMS (m/z): calculated for C₁₅H₃₀NO₂Si [$M+H$]⁺ 284.2040, found 284.2039.

$[\alpha]_D^{24}$: -4.8°, (c = 0.93, CHCl₃)

Mp: 73-75 °C.

β -lactam 27: To a solution of β -lactam **26** (1.50 g, 5.24 mmol, 1.0 equiv), enone **S4** (2.27 g, 12.3 mmol, 2.4 equiv) in degassed CH_2Cl_2 (10 mL) was added chlorodicyclohexylborane (0.524 mL, 0.524 mmol, 1.0 M in hexane, 0.1 equiv) at 25 °C. After 15 min, second-generation Hoveyda-Grubbs catalyst (0.288 g, 0.45 mmol, 0.08 equiv) was added. The reaction was heated to reflux for 16 h and then concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 0% to 80% EtOAc in hexanes) to afford the β -lactam **27** (1.57 g, 68%) as a black oil.

R_f = 0.23 (40% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 6.80 (dt, J = 15.8, 6.8 Hz, 1H), 6.13 (dt, J = 15.8, 1.6 Hz, 1H), 5.90 (s, 1H), 4.19–4.14 (m, 1H), 3.66 (td, J = 6.7, 2.2 Hz, 1H), 2.76 (dd, J = 5.3, 1.6 Hz, 1H), 2.51 (t, J = 7.5 Hz, 2H), 2.27 (dddt, J = 8.3, 6.1, 4.3, 1.7 Hz, 2H), 1.84–1.78 (m, 2H), 1.61–1.59 (m, 2H), 1.30–1.25 (m, 12H), 1.23 (d, J = 6.2 Hz, 3H), 0.87 (s, 9H), 0.87 (t, J = 7.0 Hz, 3H), 0.07 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 200.8, 168.7, 145.2, 131.1, 65.9, 64.8, 51.2, 40.8, 33.9, 32.2, 29.8, 29.8, 29.7, 29.6, 29.6, 26.1 (3C), 24.5, 23.1, 23.0, 18.3, 14.4, -3.9, -4.6.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3228, 2926, 2866, 1762, 835.

HRMS (m/z): calculated for $\text{C}_{25}\text{H}_{48}\text{NO}_3\text{Si}$ $[\text{M}+\text{H}]^+$ 438.3398, found 438.3394.

$[\alpha]_{\text{D}}^{25}$: -2.9°, (c = 0.51, CHCl_3)

***O*-silylated bicyclic β -lactam **28**:** To a solution of β -lactam **27** (1.57 g, 3.59 mmol, 1.0 equiv) in THF (224 mL) at 25 °C was added LiHMDS (1.61 mL, 1.61 mmol, 1.0 M in THF, 0.45 equiv). After 15 min, the reaction was quenched (sat. aq. NH_4Cl , 25 mL), the aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated under reduced pressure to provide a 9:1 diastereomeric ratio mixture. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 0% to 40% EtOAc in hexanes) to afford the *O*-silylated bicyclic β -lactam **28** as a colorless oil (1.05 g, 67%) Note: The TBS protected alcohol helps the separation of those two diastereomers.

R_f = 0.28 (20% EtOAc in hexanes)

^1H NMR (700 MHz, CDCl_3) δ 4.16–4.12 (m, 2H), 3.67 (ddd, J = 8.1, 5.9, 2.0 Hz, 1H), 2.73 (dd, J = 5.4, 2.0 Hz, 1H), 2.66 (dd, J = 16.4, 6.0 Hz, 1H), 2.47–2.40 (m, 4H), 2.12 (dtd, J = 12.8, 6.4, 2.9 Hz, 1H), 1.76–1.71 (m, 1H), 1.56–1.49 (m, 3H), 1.29–1.24 (m, 12H), 1.20 (d, J = 6.2 Hz, 3H), 0.87 (s, 9H), 0.87 (t, J = 7.0 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (176 MHz, CDCl_3) δ 209.0, 176.8, 66.0, 64.0, 55.0, 54.2, 48.3, 43.2, 37.7, 32.0, 30.6, 29.6, 29.6, 29.4, 29.3, 25.9 (3C), 24.0, 22.8, 22.8, 18.1, 14.3, -4.1, -4.8.

IR (Diamond-ATR, neat) $\tilde{\nu}_{\text{max}}$: 3038, 2926, 2773, 1768, 1882, 835.

HRMS (m/z): calculated for $\text{C}_{25}\text{H}_{48}\text{NO}_3\text{Si}$ $[\text{M}+\text{H}]^+$ 438.3398, found 438.3394.

$[\alpha]_D^{24}$: +43.7°, (c = 0.94, CHCl_3)

Bicyclic β -lactam 17: To a solution of *O*-silylated bicyclic β -lactam **28** (1.23 g, 2.80 mmol, 1.0 equiv) in dry THF (14 mL) was added acetic acid (0.200 mL, 3.50 mmol, 1.3 equiv) followed by TBAF (3.36 mL, 3.36 mmol, 1.0 M in THF, 1.2 equiv) dropwise at 25 °C then heated to 48 °C. After 12 h, the reaction was quenched (sat. aq. NH_4Cl , 5 mL), the aqueous layer was extracted with EtOAc (3 x 10 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO_2 , gradient elution: 12% to 100% EtOAc in hexanes) to afford the bicyclic β -lactam **20** (0.762 g, 84%) as a colorless oil.

The characterization data are consistent with **17**.

Evaluation of biological activities:

General information:

Methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MSSA/MRSA) strains were obtained from the American Type Culture Collection (ATCC: 29213, 33591) and colonies were grown on Tryptic soy agar (TSA) or Mueller-Hinton broth (MHB) at 37 °C. The *Acinetobacter baumannii* type strain ATCC 19606 was obtained from the American Type Culture Collection (ATCC) and colonies were grown on Nutrient agar (NA) or in Lennox broth base (LB) at 37 °C.

Mueller-Hinton broth (MHB, 211443-BD) and Lennox broth base (LB, Invitrogen: 12780-052) were purchased from Fisher Scientific. Tryptic soy agar (TSA, cat. # 22091) and Linezolid (cat. # P70014) were purchased from Sigma-Aldrich. All assays were run in triplicate and repeated at least two separate times for MIC assays. All compounds were dissolved in molecular biology grade DMSO as 10 mM stock solutions. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer.

Broth microdilution method for determination of minimum inhibitory concentration (MIC)

MIC was determined by broth micro-dilution according to CLSI guidelines.^x MSSA, MRSA and *A. baumannii* was grown in appropriate medium for 6-8 h; this culture was used to inoculate fresh MHB (5×10^5 CFU/mL). The resulting bacterial suspension was aliquoted (1 mL) into 1.5 mL microtubes and compound was added from a 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128 µg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. Rows 2-12 of a 96-well microtiter plate were filled at 100 µL/well from the remaining inoculated media. The samples containing test compounds and linezolid were then aliquoted (200µL) into the corresponding first row wells of the microtiter plate (two wells for each compound and two negative controls). Row 1 wells were mixed 6 to 8 times, then 100 µL was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100 µL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate. The plate was then covered and sealed with GLAD Press'n Seal® and incubated under stationary conditions at 37 °C. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed.

MIC data for batzelladine D and analogs

Strain / Compound	MIC values (μ g/mL)							
	(-)-2	(-)-2	(-)-23	(-)-19	(-)-S2	(-)-19	(-)-S2	(-)-S1
<i>S. aureus</i> 33591 (MRSA)	8	8	4	8	2	2	2	8
<i>S. aureus</i> 29213 (MSSA)	8	8	4	8	2	2	2	8
<i>A. baumannii</i> 19606	128	64	32	256	32	64	32	256

(-)-batzelladine D (2)	(+)-batzelladine D (2)	(+)-elim (23)	(-)-13-epi (19)

(-)-13-elim (S2)	(+)-13-epi (19)	(+)-13-elim (S2)	(-)-15-epi (S1)

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