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Solid-Phase Total Synthesis of Bogorol A: Stereocontrolled Construction of Thermodynamically Unfavored (*E*)-2-Amino-2-butenamide

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Supporting Information

ABSTRACT: Bogorol A [(*E*)-**1**], a potent antibiotic against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus spp.*, possesses a thermodynamically unfavored (*E*)-2-amino-2-butenamide within its linear dodecapeptide sequence. The highly efficient total synthesis of natural (*E*)-isomer (*E*)-**1** and its artificial (*Z*)-isomer (*Z*)-**1** by employing a full solid-phase strategy is reported. The (*E*)- and (*Z*)-2-amino-2-butenamide moieties were stereoselectively constructed by applying traceless Staudinger ligation on the resin. Interestingly, (*E*)- and (*Z*)-**1** showed comparable antimicrobial activity (MIC = 4 μg/mL).

Bacterial infections increasingly evade standard treatments as resistance to multiple antibiotics spreads through the microbiome. Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE), in particular, pose a global threat to public health.[1] New antibacterial lead structures that show no cross-resistance with existing antibiotics are thus urgently needed to guarantee future therapeutic efficacy.[2]

Bogorol A [(*E*)-**1**, Scheme 1] was isolated from cultures of the marine bacterium *Brevibacillus laterosporus* collected in Papua New Guinea and was reported to exhibit selective and potent activity against MRSA [MIC (minimum inhibitory concentration) = 2.5 μg/mL] and VRE (MIC = 9 μg/mL).[3] One dehydroamino acid [(*E*)-ΔAbu] and four D-amino acids (D-Orn, D-Lys, D-Leu, and D-Tyr) are incorporated within the linear dodecapeptide sequence, which is further modified by (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid (N-cap) at the N-terminus and L-valinol (C-cap) at the C-terminus. These nonproteinogenic structural units indicate the nonribosomal origin of (*E*)-**1**.[4] In addition, since the three amino groups of the side chains contribute to the net positive charge of the molecule, (*E*)-**1** is classified as a cationic peptide antibiotic. These peptide antibiotics are widespread in nature, are an integral part of the innate immune systems,[5] and have attracted much attention as possible new-generation clinically useful antibacterial agents due to their potent antibacterial activity, broad antibiotic spectrum, and low propensity for rapid emergence of resistance.[6] Although many of these peptides are known to kill bacteria by physically disrupting membrane function, the precise mode of action of (*E*)-**1** remains unknown.

The most unusual structural feature of (*E*)-**1** is the presence of thermodynamically unfavored (*E*)-2-amino-2-butenoic acid [(*E*)-ΔAbu]. The conformation of the dehydroamino acid residue is influenced by the β-substituent on a C_α=C_β double bond; consequently, the *E*/*Z* geometry of the olefin impacts the dynamic behavior, overall three-dimensional structure, and biological activity.[7,8] The characteristic dehydroamino acid residue of (*E*)-**1**, together with its important antibacterial function, motivated us to undertake the total chemical construction of both natural (*E*)-**1** and its artificial (*Z*)-isomer (*Z*)-**1**. Herein we describe a general method for the stereoselective synthesis of both (*E*)-ΔAbu and (*Z*)-ΔAbu amides and report the solid-phase total synthesis of bogorol A [(*E*)-**1**] and its isomer (*Z*)-**1**. Intriguingly, preliminary biological evaluation revealed that the geometrical isomer (*Z*)-**1** was equipotent to (*E*)-**1**.

We envisioned adopting the full solid-phase approach for the total syntheses of (*E*)- and (*Z*)-**1** in order to simplify the operations and to facilitate a future comprehensive structure–activity relationship study to search for optimized analogues (Scheme 1).[9,10] Resin-bound dodecapeptides (*E*)- and (*Z*)-**2** were thus designed as the precursors of (*E*)- and (*Z*)-**1**, respectively. In the synthetic direction, a single acidic treatment would simultaneously realize release of (*E*)- or (*Z*)-**1** from the 2-chlorotrityl resin and removal of all the acid labile protecting groups (TBS of N-cap, Boc of D-Orn, D-Lys and L-Lys, and *t*-Bu of D-Tyr) from the side chains. Protected peptides (*E*)- and (*Z*)-**2** would in turn be elongated by the combination of standard Fmoc-based solid-phase peptide synthesis (SPPS)[11] and on-resin construction of the (*E*)-ΔAbu and (*Z*)-ΔAbu amides, respectively.

The well-known thermodynamic instability of an (*E*)-α,β-dehydroamino acid residue in comparison to its (*Z*)-counterpart makes its stereoselective construction highly challenging.[12,13] In general, the 1,3-allylic strain of the (*E*)-dehydroamino acid residue is higher than that of the (*Z*)-

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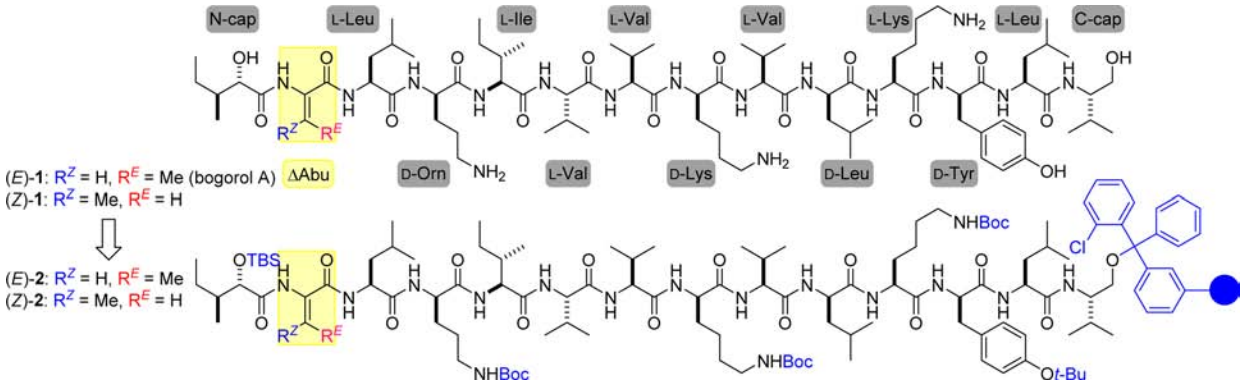
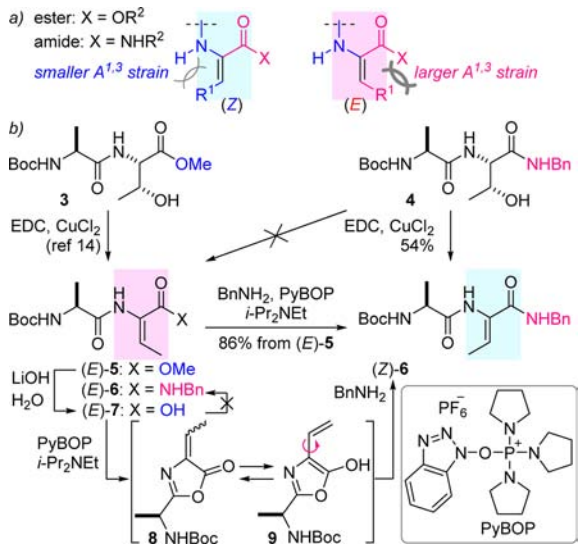
Scheme 1. Structures and Retrosyntheses of Bogorol A and Its Isomer[a]



[a]TBS = *tert*-butyldimethylsilyl; Boc = *tert*-butoxycarbonyl.

derivative and increases when the substituted nitrogen (amide: X = NHR^2) is introduced at the C-terminus in place of the less sterically bulky oxygen (ester: X = OR^2) (Scheme 2a). To develop a robust method for the stereoselective construction of the (*E*)-ΔAbu amide, the synthesis of (*E*)-**6** was pursued as a model study (Scheme 2b).

Scheme 2. Attempted Synthesis of the (*E*)-ΔAbu Amide by Dehydration[a]

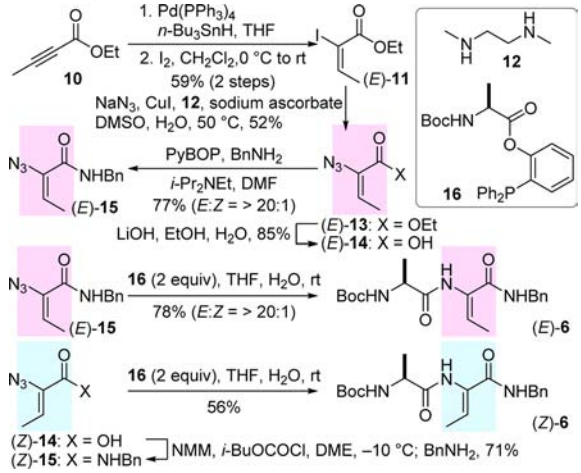


[a]EDC = 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

In accordance with Sai's report,[14] the EDC/$CuCl_2$-promoted syn-elimination of threonine methyl ester **3** selectively generated (*E*)-ΔAbu ester (*E*)-**5**. However, the reaction of threonine benzyl amide **4** under the same conditions was more sluggish, slowly generating thermodynamically stable (*Z*)-**6** as the predominant product. Conversion of ester (*E*)-**5** to amide (*E*)-**6** was also not successful. Saponification of (*E*)-**5** proceeded without isomerization, but condensation between carboxylic acid (*E*)-**7** and benzylamine only afforded undesired (*Z*)-**6**. In this reaction, the formation of the activated ester from (*E*)-**7** was presumably followed by cyclization into azlactone **8**, and equilibration between **8** and oxazole **9** resulted in geometrical isomerization.[15] Since seemingly straightforward dehydration proved to be problematic, an alternative approach was sought for the synthesis of the model compound (*E*)-**6**.

In our total syntheses of nobilamides B and D,[16] a new method for the stereoselective construction of the thermodynamically favored (*Z*)-ΔAbu ester was devised by employing traceless Staudinger ligation.[17,18] Thus, we applied this protocol to the more challenging (*E*)-ΔAbu amide (*E*)-**6** (Scheme 3).

Scheme 3. Stereocontrolled Syntheses of the (*E*)- and (*Z*)-ΔAbu Amides[a]



[a]DMSO = dimethyl sulfoxide; DMF = *N*,*N*-dimethylformamide; THF = tetrahydrofuran, NMM = *N*-methylmorpholine; DME = 1,2-dimethoxyethane.

Before doing so, alkenyl azide (*E*)-**14** was prepared by a four-step sequence from ethyl but-2-ynoate **10**. Specifically, regio- and stereoselective hydrostannation[19] of **10** and subsequent iododestannation[20] furnished alkenyl iodide (*E*)-**11**. Treatment of iodide (*E*)-**11** with NaN_3, CuI, and ligand **12** in turn afforded azide (*E*)-**13**, the ester moiety of which was hydrolyzed to generate carboxylic acid (*E*)-**14**. Then, PyBOP[21]-promoted condensation of (*E*)-**14** with benzylamine stereospecifically provided amide (*E*)-**15**. No isomerization would originate from the absence of the N-terminal carbonyl group, which would cyclize into the azlactone derivative (e.g., **8**). Traceless Staudinger ligation of the obtained azide (*E*)-**15** occurred at room temperature by simply mixing with phosphine **16**[16] in aqueous THF for 24 h, furnishing the (*E*)-ΔAbu amide (*E*)-**6** via ejection of (2-hydroxyphenyl)diphenylphosphine oxide

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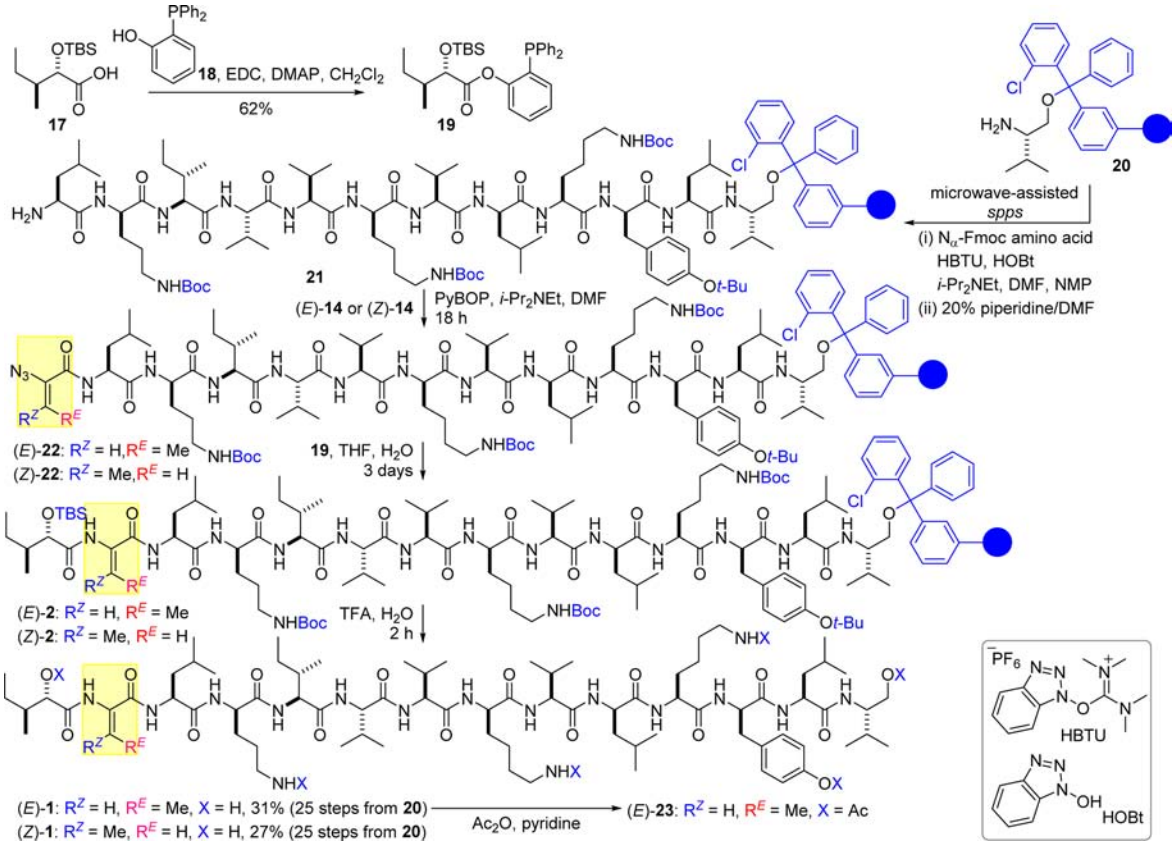
Scheme 4. Total Syntheses of Bogorol A [(*E*)-1] and Its Isomer (*Z*)-1[a]



[a]DMAP = *N*,*N*-dimethyl-4-aminopyridine; Fmoc =9-fluorenylmethyloxycarbonyl; HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt = 1-hydroxybenzotriazole; NMP = *N*-methylpyrrolidone; TFA = trifluoroacetic acid.

(78% yield). Moreover, the method was found to be applicable for the synthesis of the (*Z*)-ΔAbu amide (*Z*)-**6**. Amidation of acid (*Z*)-**14**[16] and benzylamine using NMM and *i*-BuOCOCl, and subsequent treatment of amide (*Z*)-**15** with **16**, led to stereoselective generation of (*Z*)-**6**. The results shown in Scheme 3 clarified the superiority of the traceless Staudinger approach over conventional dehydration in terms of reliable stereoselective outcomes and mild reaction conditions. Additionally, the order of stepwise amide bond formations, with the C-terminus condensation preceding the N_α-Staudinger coupling, permitted direct adoption of the method to the SPPS of the targeted (*E*)- and (*Z*)-**1**.

Prior to the full solid-phase total syntheses of bogorol A [(*E*)-**1**] and its isomer (*Z*)-**1**, N-cap **19**, the reactive substrate for the traceless Staudinger ligation, was prepared from the known carboxylic acid **17**[22] and phosphinophenol **18**[23] using EDC and DMAP (Scheme 4). Then, undecapeptide **21** was elongated from the C-cap-bound chlorotrityl resin **20** by employing Fmoc chemistry under microwave-assisted conditions.[24] Namely, 11 cycles of HBTU/HOBt[25]-mediated peptide coupling and piperidine-promoted N_α-deprotection using the component amino acid monomers [Fmoc-L-Leu-OH, Fmoc-D-Tyr(*t*-Bu)-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-D-Leu-OH, Fmoc-L-Val-OH, Fmoc-D-Lys(Boc)-OH, Fmoc-L-Val-OH, Fmoc-L-Val-OH, Fmoc-L-Ile-OH, Fmoc-D-Orn(Boc)-OH, and Fmoc-L-Leu-OH] transformed **20** into **21**. The crucial installation of (*E*)-ΔAbu within the sequence was achieved on-resin by the subsequent two steps. Acid (*E*)-**14** was attached to the N-terminus of **21** using PyBOP to produce azide (*E*)-**22**, which was treated with N-cap **19** in aqueous THF (0.029 M) for 3 days, delivering the fully protected bogorol A [(*E*)-**2**]. Lastly, subjection of (*E*)-**2** to 95% aqueous TFA simultaneously removed the *t*-Bu, Boc, and TBS groups and cleaved the peptide from the resin to release (*E*)-**1** into solution. After purification by the reversed-phase HPLC, bogorol A [(*E*)-**1**] was obtained as the single isomer in 31% total yield in 25 steps from **20**. The non-natural (*Z*)-isomer of bogorol A [(*Z*)-**1**] was constructed in the same manner by using (*Z*)-**14** instead of (*E*)-**14** [**20** → (*Z*)-**2** → (*Z*)-**1**, 27% total yield in 25 steps]. The exclusive formation of the (*E*)-ΔAbu and (*Z*)-ΔAbu residues and excellent overall yields of (*E*)- and (*Z*)-**1** demonstrated the power and generality of the traceless Staudinger approach for the assembly of complex dehydro peptides. It is noteworthy that, in contrast to the facile isomerization of the (*E*)-ΔAbu residue observed in Scheme 2, the olefin geometry of (*E*)-**1** was stable when incorporated into the sequence: isomerization between (*E*)- and (*Z*)-**1** was not detected upon separate incubation of the two isomers in aqueous buffer at 40 °C for 24 h.

Assignment of the ^{1}H and ^{13}C NMR peaks of (*E*)-**1** was problematic due to significant overlaps. Accordingly, the authenticity of (*E*)-**1** was confirmed after its derivatization into the hexaacetylated compound (*E*)-**23**.[3a] The ^{1}H and ^{13}C NMR spectra of synthetic (*E*)-**23** were fully assigned and shown to match with (*E*)-**23** derived from the isolated natural product **1**.

A preliminary antibacterial study of (*E*)- and (*Z*)-**1** was carried out using methicillin-susceptible *S. aureus* (MSSA).

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Interestingly, both geometrical isomers showed the same MIC value of 4 μg/mL. Although the exact role of the ΔAbu amide remains to be elucidated, the equipotency of natural (*E*)-**1** and artificial (*Z*)-**1** revealed the inconsequential nature of the olefin geometry for exerting bioactivity.

In conclusion, we have achieved the total synthesis and biological evaluation of antibiotic bogorol A [(*E*)-**1**] and its isomer (*Z*)-**1** for the first time. Traceless Staudinger ligation was utilized for stereoselective construction of both the thermodynamically unfavored (*E*)-ΔAbu amide of (*E*)-**1** and the favored (*Z*)-ΔAbu amide of (*Z*)-**1**. The developed method was general and robust in comparison to the previous dehydrative olefination method and enabled a high-yielding solid-phase route to the target compounds (*E*)- and (*Z*)-**1** (approximately 30% overall yields). These advantageous features indicate that the present method should have broad application to numerous peptide natural products possessing dehydroamino acid residues. A more comprehensive SAR study of (*E*)-**1** in future research will aid clarification of the structural roles of the amino acid components, elucidation of the unknown molecular mode of action, and development of new chemotherapeutic agents against MRSA and VRE.

■ ASSOCIATED CONTENT

Supporting Information

Characterization data for all new compounds and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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