## Natural Products

## Structure Determination and Total Synthesis of Bottromycin A<sub>2</sub>: A Potent Antibiotic against MRSA and VRE\*\*

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The emergence of antibiotic resistance in bacteria has been a major and increasing threat to public health worldwide. There is an urgent need for novel antibiotics to help solve the problem. In the course of our search for natural products with activity against drug-resistant bacteria, we rediscovered an antibiotic, bottromycin  $A_2$  (1; Figure 1), which was first



Figure 1. Structure of bottromycin  $A_2$  and reported optical rotation of Thia- $\beta$ -Ala and tLeu.

isolated from the fermentation broth of *Streptomyces bottropensis* by Waisvisz et al. in 1957.<sup>[1]</sup> Although **1** is known to show antibacterial activity against Gram-positive bacteria and

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The gross structure of bottromyin A2 has been established through repeated revisions since its first isolation.<sup>[4-6]</sup> The most significant structural characteristic is an amidine moiety formed through condensation of the cyclic tetrapeptide and linear tripeptide units.<sup>[6]</sup> However, there is no clear experimental data that directly demonstrates a 12-membered cyclic skeleton in the structure of 1, its cyclic structure was established solely based on CIMS analysis.<sup>[5]</sup> In addition, there is a lack of clarity concerning the configuration of tertleucine (tLeu 1: C18; tLeu 2: C25) and 3-(thiazol-2-yl)-βalanine (Thia-β-Ala-OMe: C43) residues. After degredation, the *t*Leu unit(s) derived from 1 was thought to have the L form because it was not affected by D-amino acid oxidase and showed a slight positive Cotton effect in ORD (optical rotation dispersion) curves.<sup>[7]</sup> However, the optical rotations of the natural and synthetic  $tLeu^{[8]}$  are opposite, which suggests that the natural *t*Leu is more likely to have the D form (Figure 1). In the case of Thia-β-Ala, its optical rotation was less than expected, even though natural Thia-β-Ala was proved to have the L form based on the experimental rule in ORD of  $\beta$ -amino acids.<sup>[9]</sup> Herein, we report the structure elucidation and total synthesis of 1 and therefore resolve the existing structural ambiguities.

We first undertook studies to confirm the structure of bottromycin  $A_2$  (1) by using high-resolution NMR spectroscopy (600 MHz). Our independent assignments of the proton and carbon signals of 1 were found to be compatible with those report by Kaneda.<sup>[10]</sup> We performed an HMBC experiment to establish the 12-membered cyclic skeleton in 1 (see the Supporting Information). By using a long-range delay of 125 ms that corresponds to a  $1/nJ_{CH}$  of 4 Hz, previously unreported HMBC correlations between Gly and *t*Leu 1 (1NH and 23C, 2H and 23C) were clearly observed. This is the first experimental evidence that firmly establishes the 12-membered cyclic skeleton in 1.



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We next estimated the absolute configurations at C18 and C25 by using a combination method of conformation analysis with high-temperature molecular dynamics (MD) and NMR spectroscopy (see the Supporting Information).<sup>[11]</sup> The conformation analysis was performed toward a simplified structure **2** differing in the configuration at C18 and C25 (Figure 2A) by using the CAMDAS (Conformational Ana-



**Figure 2.** A) Model structure **2** shown as the 185,255 configuration, which was determined by the conformation analysis and NMR experiments. B) Stereopairs of the superposition of the resulting two 3D structures of model structure **2**. Black and grey structures have MMFF energies of 104.5 and 115.1 kcalmol<sup>-1</sup>, respectively. These are the results of the best fit of the heavy atoms. MMFF = Merck Molecular Force Field

lyzer with Molecular Dynamics and Sampling) 2.1 program.<sup>[12]</sup> The NMR experiments with **1** provide the total 32 requisite constraints, which consist of 2 dihedral and 30 distance constraints. The RMS deviations for all configurations generated by CAMDS were subsequently calculated to determine which configurations can adopt conformers accommodating the NMR constraints. The conformers that satisfied all experimental constraints were found in only one configuration. The process described above led to a set of two energy-minimized 3D structures of model structure **2**, which indicates the 18*S*,25*S* configurations (Figure 2B). We therefore established (18*S*,25*S*)-**1** as the target of the following total synthesis.

Prior to the start of our total synthesis, we performed a degradation study of **1** to obtain additional information about the configuration at C18, C25, and C43 (Scheme 1). Pyrolysis of **1** in MeOH at 130 °C was carried out in a sealed tube to provide cyclic product **3** and dipeptide **4** in 89% and 95% yield, respectively. In addition, treatment of **1** with excess NaBH<sub>4</sub> resulted in reductive cleavage of an amide bond between the Gly and MePro units to give amino alcohol **5**. The cyclic product **3** was obtained as an inseparable mixture of diastereomers at C25 owing to keto/enol tautomerization at the imidazolone moiety. This tautomerization suggested that the reported hydrolysis of **1** might cause racemization of *t*Leu via the imidazolone path a). We therefore anticipated that **4** and **5** would be useful synthetic targets to help



**Scheme 1.** Reagents and conditions: a) MeOH, 130 °C, in a sealed tube, 3 h, **3**; 89%, **4**; 95%; b) NaBH<sub>4</sub>, MeOH, 0 °C, 30 min, 63%.

determine the configuration at C18, C25, and C43. We planed to confirm the absolute structure by tracing the natural degradation products **4** and **5** to our goal, the total synthesis of **1**.

Determination of the configuration at C43 required enantiomers of Thia- $\beta$ -Ala that were susceptible to epimerization.<sup>[9]</sup> Our stereoselective synthesis of Thia- $\beta$ -Ala-OMe **12** was performed by applying chiral sulfinamide chemistry developed by the research groups of Davis and Elmann,<sup>[13]</sup> as shown in Scheme 2. Condensation of ( $S_S$ )-*para*-toluene sulfinamide **6** with 2-formylthiazole **7** afforded sulfinimine **8** in 90% yield.<sup>[14]</sup> The Mannich reaction of **8** with allyl methyl malonate **9** quantitatively gave adduct **10** as a 1:1 mixture of  $\alpha$ -diastereomers, which was subjected to palladium-catalyzed decarboxylation to give methyl ester **11** as a single diastereomer in 76% yield.<sup>[15]</sup> Finally, removal of the sulfinyl group afforded (+)-(43*R*)-**12** in quantitative yield (>99% *ee*). The absolute configuration of (+)-(43*R*)-**12** was later confirmed by X-ray crystallographic analysis of (43*R*)-**15**.<sup>[17]</sup> The enan-



Scheme 2. Reagents and conditions: a) 7,  $Cs_2CO_3$ ,  $CH_2Cl_2$ , 40°C, 1 h, 90%; b) 9, NaHMDS, THF, -78°C, 30 min, quant.; c) [Pd(PPh\_3)\_4], HCO\_2H, Et\_3N, THF, reflux, 2 h, 76%; d) TFA, MeOH, 0°C, 3 h, quant. HMDS = hexamethyldisilazane, TFA = trifluoroacetic acid.

## Communications

tiomer (-)-(43S)-12 was synthesized in the same manner using  $(R_s)$ -tert-butyl sulfinamide 13.

Each enantiomer of **12** was condensed with azido acid **14**<sup>[16]</sup> using PyBOP and gave azido dipeptides **15**, respectively (Scheme 3). At this stage, the absolute configuration at the C43 position of (43R)-**15** was confirmed as the *R* configuration by X-ray crystallographic analysis (see the Supporting Information).<sup>[17]</sup> Finally, reduction of the azido group afforded a set of diastereomers **4**. We then compared



**Scheme 3.** Reagents and conditions: a) **14**, PyBOP, *i*Pr<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, RT, 1 h, (43*R*)-**15**, quant.; (43*S*)-**15**, quant.; b) H<sub>2</sub>, 10% Pd/C, MeOH, RT, 1 h, (43*R*)-**4**; 97%; (43*S*)-**4**; 93%. PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

<sup>1</sup>H NMR spectroscopic data between synthetic and natural samples to assign the configuration at C43 (Figure 3). The <sup>1</sup>H NMR spectrum of the natural degradation product matched that of (43R)-4, whereas the chemical shift of the methylene group at C49 in (43S)-4 was substantially different.



*Figure 3.* Comparison of <sup>1</sup>H NMR spectra of diastereomers at C43 for **4**.



Scheme 4. Reagents and conditions: a) 16, HBTU,  $iPr_2NEt$ ,  $CH_2Cl_2/DMF$ , RT, 2 h, quant.; b)  $4 \times HCl/dioxane$ , RT, 30 min, quant.; c) 18,  $Hg(OTf)_2$ , 2,6-lutidine,  $CH_3CN$ , RT, 30 min, 96%; d)  $N_2H_4$ · $H_2O$ , EtOH, reflux, 2 h; e) 20, HBTU,  $iPr_2NEt$ ,  $CH_2Cl_2/DMF$ , RT, 2 h, 89% (over 2 steps); f) TFA/  $CH_2Cl_2$ , RT, 3 h; g) 22, HBTU,  $iPr_2NEt$ ,  $CH_2Cl_2/DMF$ , RT, 2 h, quant. (over 2 steps); h) TBAF, THF, RT, 3 h; i)  $4 \times HCl/dioxane$ , RT, 30 min, 68% (over 2 steps from 23); j) Jones reagent (15 equiv), acetone, 0°C, 4 h, 55% (over 2 steps from 23); k)  $4 \times HCl/dioxane$ , RT, 30 min; l) EDCI·HCl (10 equiv),  $iPr_2NEt$  (4 equiv),  $CH_2Cl_2$ , RT, 4 h, 51% (over 2 steps). Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide, EDCI = *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide, HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*'. tetramethyluronium hexafluorophosphate, Pht = phthaloyl, TBAF = *tetra*-*n*-butylammonium fluoride, TBDPS = *tert*-butyldiphenylsilyl, Tf = trifluoromethanesulfonyl.

Thus <sup>1</sup>H NMR analysis indicates that the configuration at C43 of **1** is the *R* configuration.

With the dipeptide (43R)-4 in hand, we then undertook the synthesis of amino alcohol 5 to confirm the remaining configurations at C18 and C25, which had been estimated by the conformation analysis (Scheme 4). The dipeptide (43R)-4 was condensed with Boc-tLeu-OH 16, and subsequent removal of the Boc group provided tripeptide 17. In our total synthesis intermolecular amidine formation was a key step, and we examined mercury-mediated condensation between an amine and a thioamide. We found that Nphthaloyl thioamide 18 could be smoothly coupled with 17 by the combination of Hg(OTf)<sub>2</sub> and 2,6-lutidine to furnish amidine 19 in 96% yield. Removal of the phthaloyl group and subsequent condensation with Boc-Val-OH 20 afforded 21 in 89% yield. After removal of the Boc group of 21, the resulting amine was coupled with Boc-MePro-OH 22<sup>[18]</sup> to give compound 23 in quantitative yield. Finally, removal of the TBDPS and Boc groups afforded (18S,25S,43R)-5 in 68% yield. Optical rotation, <sup>1</sup>H NMR spectroscopic, and HRMS data of the synthetic compound matched those of the degradation product derived from the natural product. From this result, we concluded that the complete structural assignment of 1 comprises 18S, 25S, and 43R configurations.

Next, we turned to completion of the total synthesis. Removal of the TBDPS group and subsequent Jones oxidation afforded carboxylic acid **24** in 55% yield from **23**. After removal of the Boc group, and macrolactamization of **25** with an excess amount of EDCI (10 equiv) and *i*Pr<sub>2</sub>NEt (4.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mM) provided **1** in 51% yield. The spectroscopic data ( $[a]_{D}$ , <sup>1</sup>H and <sup>13</sup>C NMR, IR, and HRMS) of the synthetic material were identical to those of the natural product. Furthermore, the synthetic sample of **1** showed antibacterial activity against MRSA and VRE.

In conclusion, we have achieved the first total synthesis of bottromycin  $A_2$  and confirmed its absolute structure. This accomplishment will end any uncertainty over the structure of the compound and offers significant promise for development of new and urgently required antibiotics.

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