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Total Synthesis and Antibacterial Investigation of Plusbacin A_3

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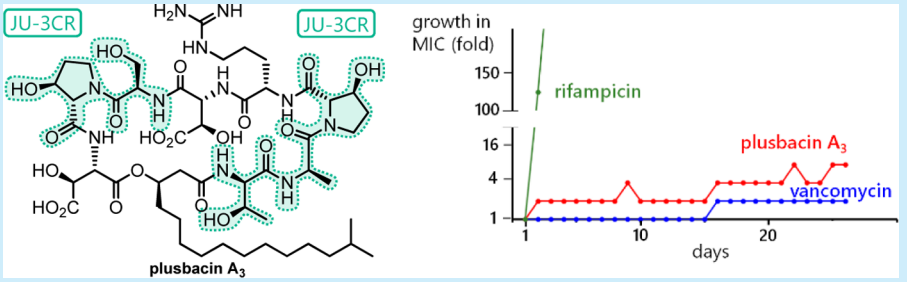
Supporting Information



ABSTRACT: The total synthesis of plusbacin A_3 (**1**) has been accomplished using a solvent-dependent diastereodivergent Joullié–Ugi three-component reaction (JU-3CR) as a key step. Two *trans*-3-hydroxy-L-proline residues were constructed by combining the JU-3CR with a convertible isocyanide strategy. Subsequent peptide coupling and macrolactamization afforded plusbacin A_3. Investigating the antibacterial activity of **1** compared with that of its dideoxy analogue revealed that the *threo*-β-hydroxyaspartic acid residues are essential for antibacterial activity. Notably, there is a low potential for the development of resistance in *S. aureus* against plusbacin A_3.

The emergence of bacterial resistance to newly developed drugs is a severe and ongoing global threat to current therapies against infectious diseases. An important criterion for selecting among potentially promising natural-product antibacterial agents is their potential to be less susceptible to the development of resistance. Plusbacin A_3 (**1**), which was isolated from *Pseudomonas* sp. PB-6250 contained in a soil sample collected from Okinawa Island, Japan,[1] is a representative cyclic lipodepsipeptide antibiotic, other examples of which are empedopeptin[2] and tripropeptins[3] (Figure 1). This class of antibiotics exhibits potent antibacterial activity against a wide range of Gram-positive bacteria. The mode of action of **1** is anticipated to be inhibition of bacterial cell-wall biosynthesis via Ca^{2+}-dependent binding to lipid II, which is the final precursor of peptidoglycan, resulting in the cessation of polymerization.[4] Considering the low bacterial resistance against vancomycin, the mode of action targeting nonprotein precursors in peptidoglycan biosynthesis promises characteristics of new antibacterial agents that may be less susceptible to the development of resistance, although cyclic lipodepsipeptides' susceptibility to resistance, including **1**, has not been investigated yet. In addition, dual inhibition of teichoic acid and peptidoglycan biosynthesis has also been reported for **1**,[5] which exhibits potent antimicrobial activity against drug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) (MIC 0.1–3.13 μg/mL).[1,6] Therefore, **1** possesses a mode of action different from those of existing antibacterial drugs and is expected to be a promising lead in novel antibacterial drug discovery. In its chemical structure, **1** contains (*R*)-3-hydroxyisohexadecanoic acid and five nonproteinogenic amino acids: D-*allo*-threonine, two *trans*-3-hydroxy-L-prolines [Pro(3-OH)], and L- and D-*threo*-β-hydroxyaspartic acid [Asp(β-OH)]. The total synthesis of **1** has been reported by VanNieuwenhze et al.[7] via a conventional peptide coupling using *trans*-Pro(3-OH) as a starting material. Construction of the Pro(3-OH) residue, which is a key element of **1**, requires a number of steps,

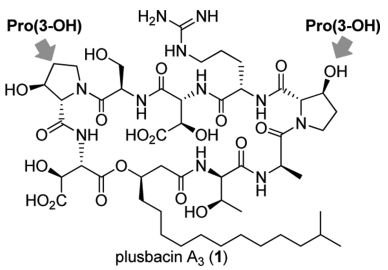


Figure 1. Chemical structure of plusbacin A_3 (**1**).

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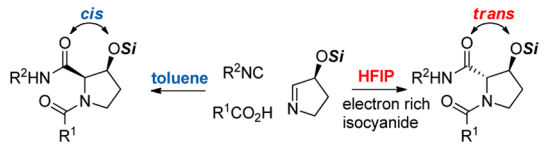
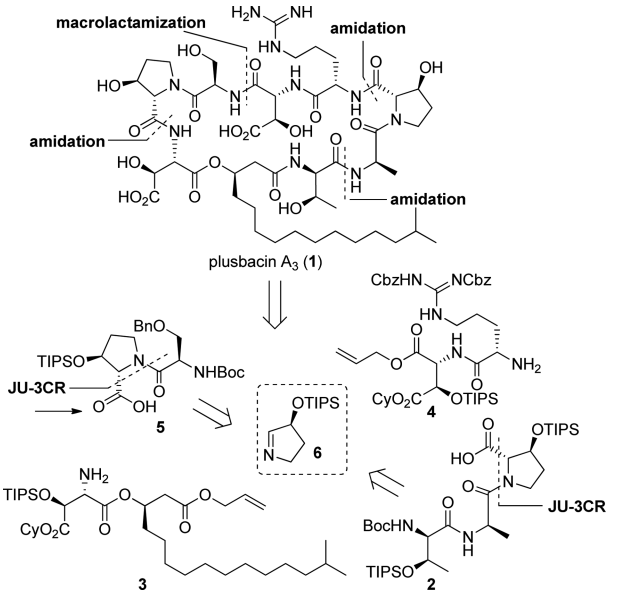
although several synthetic methods have been developed.[8] We have developed a solvent-dependent diastereodivergent Joullié–Ugi three-component reaction (JU-3CR)[9] that can provide the desired *trans*-Pro(3-OH) derivatives in a single step with concurrent modifications at both the *C*- and *N*-termini (Scheme 1).[10] In this reaction, a *trans* isomer was obtained

Scheme 1. Solvent-Dependent Diastereodivergent JU-3CR



when 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was used as a solvent, whereas a *cis* isomer was the predominant product in toluene. Moreover, we found that the solvent effect of HFIP became stronger when using more nucleophilic isocyanides. Herein, we describe the total synthesis of **1** featuring our diastereodivergent JU-3CR. The antibacterial activity of **1** and its deoxy analogue and their susceptibility for drug resistance in *S. aureus* were evaluated. Macrocycle **1** was retrosynthetically disconnected into four units, namely, **2** and **5** containing the Pro(3-OH) residue at the *C*-terminus, **3** containing a lipid chain, and **4** (Scheme 2). Assembling these four units (**2**–**5**)

Scheme 2. Retrosynthetic Analysis of Plusbacin A_3 (1)



could afford a precursor for macrolactamization. The synthesis of **2** and **5** was planned to proceed through the JU-3CR using five-membered cyclic imine **6** and a convertible isocyanide.[11]

First, the synthesis of **3**, **4**, and **17** is depicted in Scheme 3. The lipid side chain of known *β*-lactone **7**[12] was elongated via olefin cross metathesis with 2-methylbut-3-en-2-ol to afford allyl alcohol **8**. After dehydrating **8**, the resulting diene was hydrogenated, and *β*-lactone **9** was hydrolyzed to provide optically pure (*R*)-3-hydroxyisohexadecanoic acid. Then, the carboxy group was protected using allyl bromide to afford alcohol **10**. Known alcohol **11**,[13] which was prepared from (*R*)-Garner's aldehyde, was protected with a TIPS group, and the terminal olefin was converted to the aldehyde by ozonolysis. Further oxidation produced the carboxylic acid in 84% yield over three steps. Then, the carboxylic acid was reacted with 3-bromocyclohexene to give cyclohexenyl ester **12**. Pd-fibroin complex (Pd-Fib)[14] was found to be an efficient hydrogenation catalyst to suppress the hydrogenolysis of the cyclohexenyl ester moiety, and the corresponding cyclohexyl ester was provided in 85% yield. Removal of the isopropylidene group and simultaneous oxidation of the liberated alcohol were carried out under Jones' oxidation conditions, providing the protected L-Asp(*β*-OH) (**13**).[7] The condensation between **10** and **13** afforded a depsipeptide in 77% yield, and then the Boc group was cleaved under acidic conditions to afford **3**. The carboxylic acid of ***ent*-13**, which was prepared by the same procedure as **13** from (*S*)-Garner's aldehyde, was protected with an allyl group. After the removal of the Boc group, the liberated amine was condensed with Boc-Arg(Cbz)$_2$-OH to provide dipeptide **14**. Then, cleavage of the Boc group quantitatively afforded amine **4**. Protecting *allo*-D-Thr (**14**) with the Boc group followed by condensation with D-Ala-OBn gave the corresponding dipeptide. The hydroxy group was further protected with TIPS to give dipeptide **16** in 97% yield over three steps, and hydrogenolysis of the benzyl group furnished carboxylic acid **17**.

The key strategy for constructing Pro(3-OH) residues **2** and **5** with the diastereoselective JU-3CR is shown in Scheme 4. As an initial approach, the JU-3CR for the synthesis of **2** or **5** was examined in HFIP using 2-isocyanophenyl acetate,[11a] which was used as a conventional convertible isocyanide; however, these conditions gave undesirable diastereoselectivity (*trans*/*cis* = 32/68; details are described in the Supporting Information). From our previous study on the JU-3CR,[10] the observed *cis* selectivity was presumed to be attributed to the high *s* character of the sp^2 carbon atom attached to the isocyano group in 2-isocyanophenyl acetate, and an electron-rich isocyanide was expected to preferentially provide *trans* selectivity. To improve the *trans* selectivity, convertible isocyanide **18**,[11b] which was developed by Fukuyama and Kan, was used instead of 2-isocyanophenyl acetate because the *tertiary* alkyl group in **18** has a stronger electron-donating inductive effect. As expected, the JU-3CR between **6**, **17**, and **18** in HFIP favorably produced the desired *trans* isomer ***trans*-19** as a major product in 49% yield and the *cis* isomer ***cis*-19** in 29% yield (*trans*/*cis* = 62/38). The secondary amide moiety in ***trans*-19**, which was derived from isocyanide **18**, was subsequently converted to the *N*-acyloxazolidinone **20**,[11b] and then hydrolysis provided carboxylic acid **2**. The other unit **5** was synthesized by a strategy similar to the one used to synthesize **2**, and the JU-3CR between **6**, **18**, and Boc-D-Ser(OBn)-OH (**21**) gave the desired ***trans*-22** (51%) as well as ***cis*-22** (37%). The sequential formation of the corresponding oxazolidinone from ***trans*-22** followed by hydrolysis provided the desired carboxylic acid **5**.

The completion of the total synthesis is shown in Scheme 5. Peptide coupling between **3** and **5** provided depsipeptide **23** in 88% yield, and subsequent cleavage of the allyl group gave carboxylic acid **24** in quantitative yield. The condensation of **2** and **4** afforded the corresponding pentapeptide **25** in 90% yield, and the Boc group was removed to afford amine **26**. Carboxylic acid **24** was condensed with amine **26** to obtain linear peptide **27** in 64% yield. Removal of the protecting groups on the *N*- and *C*-termini of **27** gave the precursor amino acid, which was successfully cyclized upon treatment with EDCI, HOAt, and ^{i}Pr$_2$NEt in THF to provide fully protected plusbacin A_3 in 60% yield over three steps. Finally, global deprotection with anhydrous HF in the presence of anisole at −78 to 0 °C

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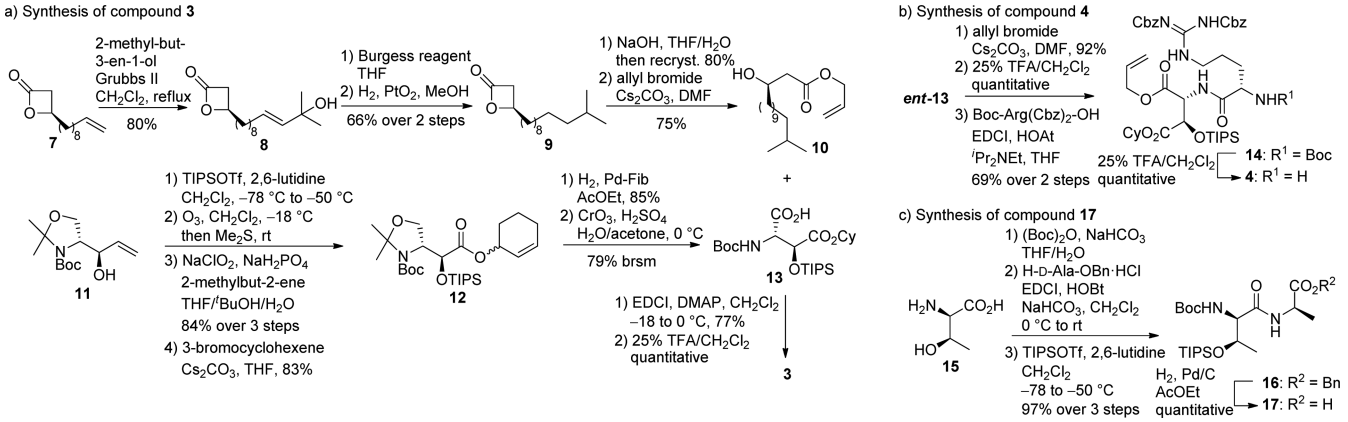
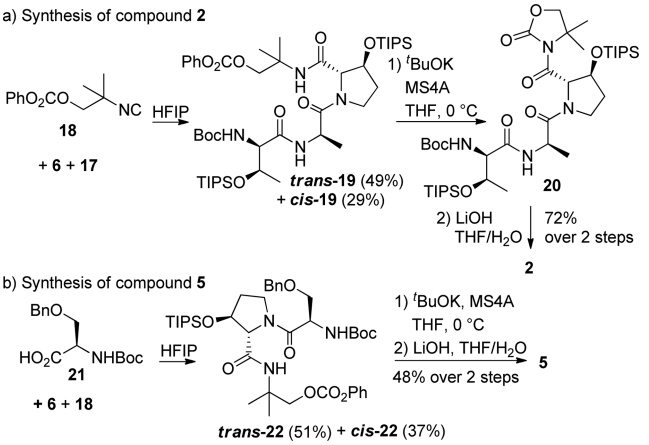
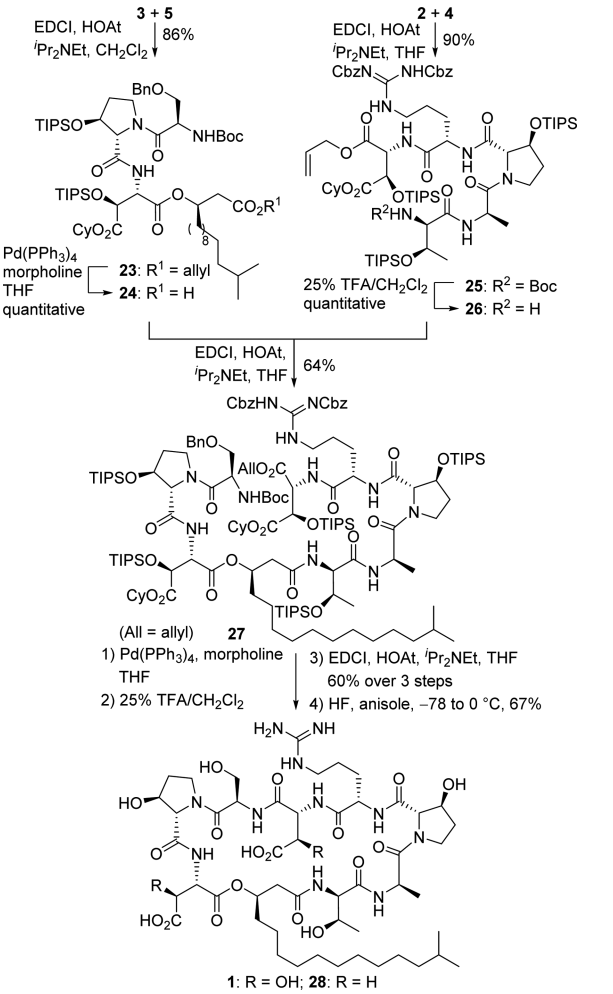
Scheme 3. Synthesis of Unit Compounds 3, 4, and 17



Scheme 4. Synthesis of Compounds 2 and 5 via JU-3CR



Scheme 5. Completion of the Total Synthesis of Plusbacin A_3



afforded **1** in 66% yield after purification through reversed-phase HPLC. The analytical data for synthetic **1** were in good agreement with the previously reported data.[7] A dideoxy analogue **28**, in which the L- and D-Asp(β-OH) groups in **1** were replaced with L- and D-Asp, respectively, was also prepared in a manner similar to the synthesis of **1** (the preparation of **28** is described in the Supporting Information).

The antibacterial activities of **1** and **28** were evaluated (Table 1). Synthetic **1** showed antibacterial activity against methicillin-sensitive *S. aureus* (MSSA, Smith and MSSA1) and MRSA (MR-6) with MIC values of 1−2 μg/mL. However, **28**, which lacks the two hydroxy groups from the Asp(β-OH) residues, did not exhibit any activity. The CD spectra of **1** and **28** indicated a three-dimensional conformational change that might be caused by altered intramolecular hydrogen bond networks (Figure S3). The lack of these two hydroxy groups in **1** induced a dramatic conformational change in the peptide backbone, resulting in a loss of binding to its target and ultimately causing a reduction in antibacterial activity. Next, we assessed resistance acquisition by *S. aureus* against **1**. Methicillin-sensitive *S. aureus* (Smith) was treated with sub MIC levels of **1** over 25 days, and an increase of the MIC values was measured by a direct comparison with vancomycin and rifampicin as controls (Figure 2). Under the conditions where a 32 000-fold increase in the MIC was observed after 2 days for rifampicin, the resistance against **1** was only twice as much as that of vancomycin, clearly indicating that **1** is much less susceptible to drug resistance. The genome sequence analysis of

Table 1. Minimum Inhibitory Concentrations (μ/mL) of 1 and 28 against *S. aureus*

	strain		
compd	Smith	MR-6	MSSA1
1	2	1	2
28	–	>128	>128

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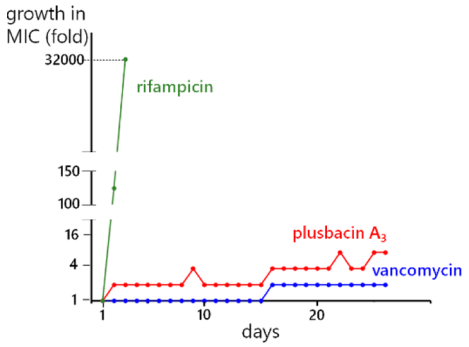

Figure 2. Resistance acquisition during serial passaging in the presence of sub-MIC levels of antimicrobials. The *x* axis is the number of days, and the *y* axis is the number of folds of MIC during passaging.

plusbacin A_3-resistant strains obtained from the resistance acquisition experiments (three strains) was performed, and mutations in proteins involved in bacterial cell-wall biosynthesis were found (Figure S4). In particular, a single mutation in VraE, which is linked to bacitracin and nisin resistance,[15] might be involved in plusbacin A_3 resistance. Nisin is a known binder to lipid II; thus, these results support the proposed mechanism of **1**.

In conclusion, we have achieved the total synthesis of plusbacin A_3 (**1**) as well as its dideoxy analogue **28** via a JU-3CR with a five-membered cyclic imine and convertible isocyanide **18**. In particular, the stereochemical outcome was adjusted by the electron density of the isocyanide building block. Evaluation of the antibacterial activity and analysis of the structure via CD suggested that the hydroxyl groups of the Asp(β-OH) residues in **1** are required to maintain its antibacterial activity. Furthermore, it was revealed that plusbacin A_3 induces only low-level resistance. These results revealed several promising properties of **1**, corroborating its potential as a lead compound for novel antibacterial agent development and mechanistic studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01629.

Experimental details; ^{1}H, ^{13}C NMR and mass spectra (PDF)

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Notes

The authors declare no competing financial interest.

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