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First Total Syntheses of Beauvericin A and *allo*-Beauvericin A

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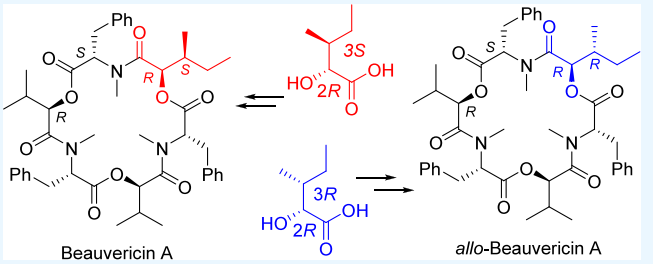
ABSTRACT: The first total syntheses of beauvericin A and *allo*-beauvericin A were achieved. *N*-Methyl-L-phenylalanine, (2*R*)-hydroxylvaleric acid, and (2*R*,3*S*)- or (2*R*,3*R*)-2-hydroxy-2-methylpentanoic acid were linked and cyclized to form the target natural products. The structure of synthetic beauvericin A was confirmed by X-ray crystallographic analysis. NMR data of the synthetic beauvericins were identical with those of the reported natural products. These results secure the structures of natural products, as originally proposed in the isolation studies.



INTRODUCTION

Beauvericins (BEAs) are a class of cyclohexadepsipeptide natural products and have attracted much attention due to their unique structural features and biological activities including antimicrobial, insecticidal, anthelmintic, herbicidal, antihaptotactic, anticholesterol, and anticancer activities (Figure 1A).[1] BEA (**1**) isolated from *Beauveria bassiana* in 1967 is the first example of BEAs.[2] The structure of **1** is characterized by the cyclic depsipeptide with C3 symmetry in which L-*N*-methyl phenylalanine (MePhe, **3**) and (2*R*)-hydroxylvaleric acid (Hiv, **4**) are repetitively linked. The absolute structures of **3** and **4** were proposed by the chemical degradation of **1** and analysis of the degradation products. Hydrolysis of **1** gave **3** and **4**. The optical rotatory dispersion spectra of the resulting **3** and **4** were compared with those of the authentic samples, suggesting the absolute stereochemistry of MePhe (**3**) and Hiv (**4**) to be 2*S* and 2*R*, respectively. The cyclic structure of **1** was also confirmed by total synthesis.[3]

Several natural and artificial beauvericin congeners have been reported.[1] Beauvericin A (BEAA, **2a**) and *allo*-beauvericin A (*allo*-BEAA, **2b**) are representative examples. BEAA (**2a**) is a natural congener of **1** isolated from the culture broth of *B. bassiana* in 1995 (Figure 1).[4] *allo*-BEAA (**2b**) is an artificial congener produced by the precursor-directed biosynthesis method feeding of L-*allo*-isoleucine (**7b**) to a fermentation broth of *Paecilomyces tenuipes* BCC 1614 (Figure 1B).[5] BEAAs **2ab** consisted of MePhe (**3**), Hiv (**4**), and 2-hydroxy-3-methylpropionic acids (Hmps, **5ab**). The structures of **5ab** were proposed to be (2*R*,3*S*)-Hmp (**5a**) for **2a** and (2*R*,3*R*)-Hmp (**5b**) for **2b** based on the hypothetical biosynthetic pathway.[4,5] Previous biosynthetic studies of (2*R*)-Hiv (**4**) showed that **4** was generated from L-valine (**6**) via 2-keto-3-methylbutylic acid (Figure 1B).[6] Similarly, it was proposed that (2*R*,3*S*)-Hmp (**5a**) and (2*R*,3*R*)-Hmp (**5b**) were presumably biosynthesized from naturally occurring L-isoleucine (**7a**) and L-*allo*-isoleucine (**7b**), respectively.

In this study, we report the first syntheses of **2ab**. NMR data of synthetic **2ab** were identical with those of the reported data. These results secure the structures of **2ab** as originally proposed in the isolation studies. Only a small structural difference appears in the Hmp moieties of **2ab**. To determine the stereochemistry of **2ab**, total synthesis using stereochemically defined synthetic methods would be one of the useful and powerful approaches. In addition, the structure determination of **2ab** provides benefits in consideration of the structure−activity relationship of beauvericins. Beauvericins are mycotoxin and often result in food poisoning.[1] Analysis and monitoring of beauvericins in food have become an important subject in terms of the engagement of risk assessment to human and animal health. In this context, synthetic samples of **2ab** would be useful tools for analytical studies.

RESULTS AND DISCUSSION

Retrosynthetic analyses of BEAA (**2a**) and *allo*-BEAA (**2b**) are depicted in Scheme 1. These target molecules would deliver from acyclic precursors **8ab**, which are disconnected to three fragments, **9**, **10a**, and **10b**. These fragments would be prepared by esterification of commercially available *N*-Fmoc-MePhe-OH (**11**) with Hiv-OBn (**12**),[3a] (2*R*,3*S*)-Hmp-OBn (**13a**),[7] or (2*R*,3*R*)-Hmp-OBn (**13b**).[8]

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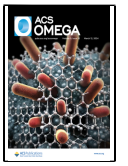


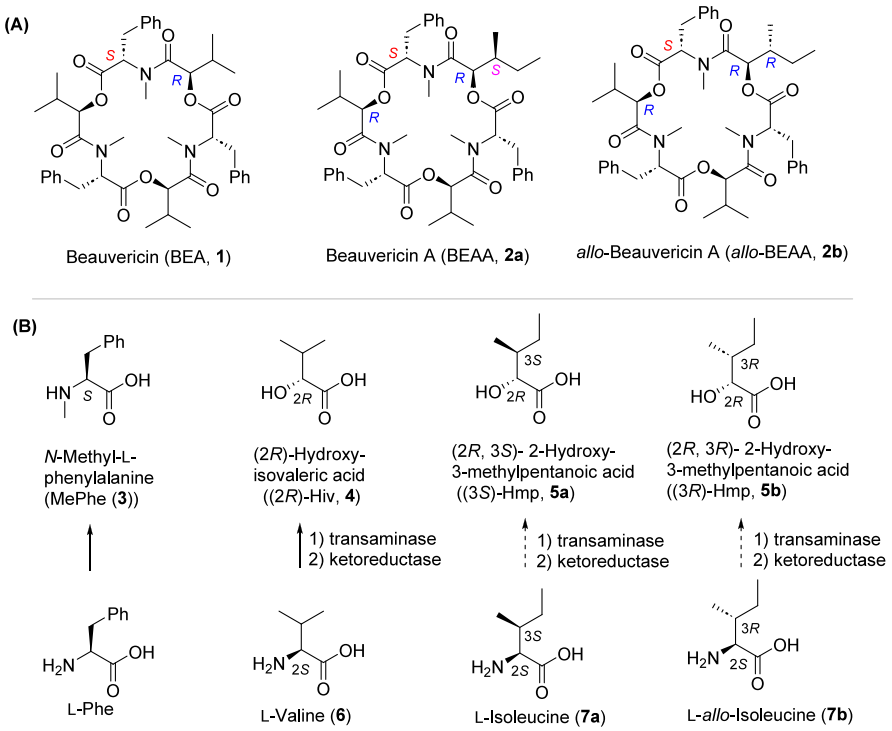
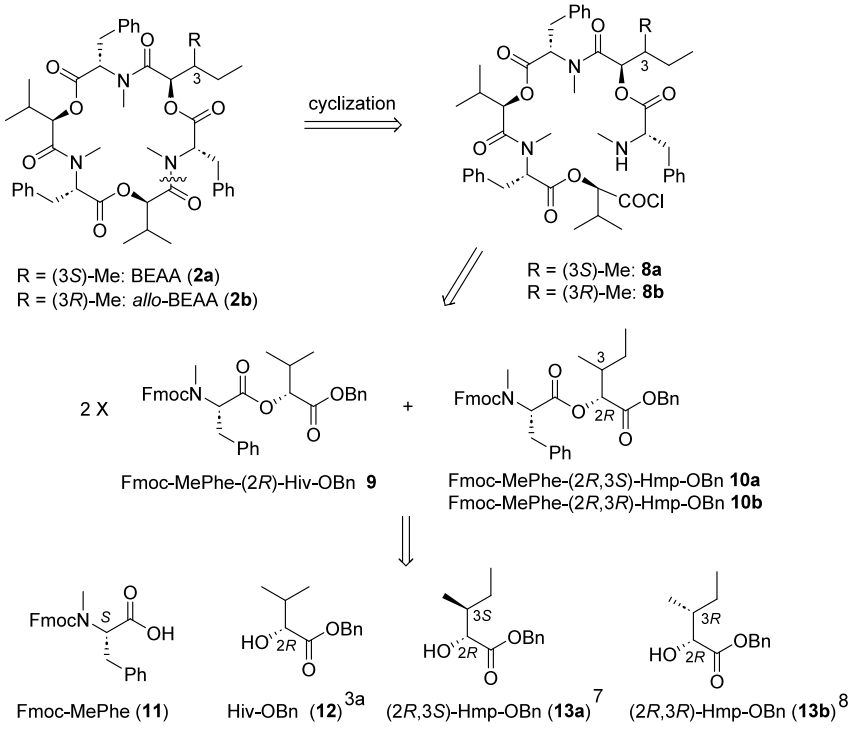


Figure 1. (A) Structures of beauvericin (BEA, **1**), beauvericin A (BEAA, **2a**), and *allo*-beauvericin A (*allo*-BEAA, **2b**). (B) Structures of the amino acid unit MePhe (**3**), 2-hydroxyacid units (2*R*)-Hiv (**4**), (2*R*,3*S*)-Hmp (**5a**), and (2*R*,3*R*)-Hmp (**5b**). (2*R*)-Hiv (**4**) is biosynthetically provided from L-valine (**6**) with the formal inversion of the (*S*)-amino acid stereochemistry of **6**.[6] Proposed biosynthesis of (2*R*)-**5ab** from L-amino acids **7ab** is done according to the biosynthesis of **4** from L-valine (**6**).

Scheme 1. Retrosynthesis of BEAA (2a) and *allo*-BEAA (2b)



N-Fmoc-MePhe-OH (**11**) was condensed with Hiv-OBn (**12**)[3a] using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of 4-dimethylaminopyridine (DMAP) to give **9** in 78% yield (Scheme 2). In a similar manner, (2*R*,3*S*)-Hmp-OBn (**13a**)[7] and (2*R*,3*R*)-Hmp-OBn (**13b**)[8] were coupled with **11** to give **10a** and **10b**, respectively.

Total synthesis of BEAA (**2a**) was achieved by coupling of **9** and **10a** (Scheme 3). Benzyl ester **9** was converted to acid **14** by hydrogenation in the presence of Pd/C under a hydrogen

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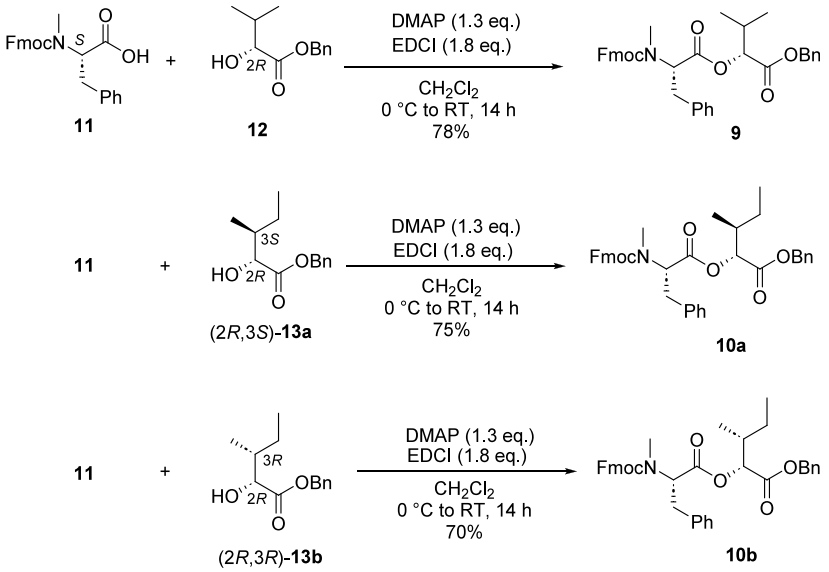
Scheme 2. Synthesis of 9 and 10ab



atmosphere. Treatment of **14** with 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez's reagent)[9] provided acid chloride **15**, which was reacted with amine **16** prepared from **9** in the presence of diisopropylethylamine (DIPEA) to give **17** in 76% yield. In a similar manner, acyclic hexadepsipeptide **21a** was prepared by coupling of acid chloride **20a** prepared from (2*R*,3*S*)-Hmp-OBn (**10a**) and amine **18** prepared from **17** in 78% yield. Sajiki et al.,[10] reported the mild removal of that *N*-Fmoc under the hydrogenation reaction condition in the presence of acetonitrile.[9] Under the condition, *N*-Fmoc and Bn protecting groups of **21a** were simultaneously removed to provide a free amino acid, which was treated with Ghosez's reagent to give acid chloride **8a**. The resulting compound **8a** was treated with DIPEA to furnish BEAA (**2a**). The structure of **2a** was unambiguously determined by X-ray crystallographic analysis (Figure 2).[11] In a similar manner, *allo*-BEAA (**2b**) was synthesized by coupling of Fmoc-MePhe-OH (**11**), Hiv-OBn (**12**), and (2*R*,3*R*)-Hmp-OBn (**13b**), followed by cyclization. ^{1}H and ^{13}C NMR data of the synthetic **2ab** are identical with those of the naturally occurring **2a**[3] and artificial biosynthetic product **2b**[4] synthesized by the precursor-directed biosynthesis, respectively (Tables 1 and 2). These results secure the originally proposed structures of BEAA (**2a**) and *allo*-BEAA (**2b**), which possess (2*R*,3*S*)-Hmp (**5a**) and (2*R*,3*R*)-Hmp (**5b**), respectively.

In summary, we have achieved the first total syntheses of BEAA (**2a**) and *allo*-BEAA (**2b**) starting from stereochemically defined staring materials **11**, **12**, and **13ab**. Comparison of NMR data of synthetic **2ab** was identical with those of the reported data described in the isolation studies. These results confirmed stereochemistry of the Hmp moieties of **2ab** and provided additional supports for the biosynthetic pathway of Hmps **5ab** where L-isoleucine (**7a**) and L-*allo*-isoleucine (**7b**) were biosynthetically transformed to **5ab** via the formal inversion of the stereochemistry. It was reported that **2a** and **2b** displayed antibacterial activities against *Mycobacterium tuberculosis* (MIC, 1.6 and 0.8 μg/mL).[5] We preliminarily tested the potency of antibacterial activity of **2a** against *Mycobacterium smegmatis*. However, **2a** did not show potent activities at >10 μg/mL. Further biological studies are ongoing in our laboratory.

EXPERIMENTAL SECTION

General Experimental Procedures. FTIR spectra were measured on a JASCO FT/IR-6200 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE III HD 400 (400 MHz) or JEOL JNM-ECZ 400S (400 MHz) spectrometer. Chemical shifts for the ^{1}H NMR spectra were reported relative to $CHCl_3$ (δ 7.26) in $CDCl_3$. ^{13}C NMR spectra were recorded on a Bruker AVANCE III HD 400 (101 MHz) or JNM-ECZ 400S spectrometer. Chemical shifts for ^{13}C NMR spectra were reported relative to $CHCl_3$ (δ 77.0) in $CDCl_3$. High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-T100LP instrument for electrospray ionization (ESI). X-Ray crystallographic analysis was performed on a Rigaku AFC11/Saturn 724+ CCD diffractometer with monochromated Mo-$K\alpha$ radiation (λ = 0.710747 Å). Optical rotations were taken on a JASCO P-1030 polarimeter with a sodium lamp (D line) using $CHCl_3$ or MeOH of spectrochemical analysis grade. Melting points were determined with Yanaco MP-21 melting point apparatus. All reactions were monitored by thin-layer chromatography (TLC), which was performed with precoated plates (silica gel 60 F-254, 0.25 mm thickness, manufactured by Merck). TLC visualization was accompanied using a UV lamp (254 nm) or a charring solution (ethanolic phosphomolybdic acid, ethanolic *p*-anisaldehyde, and butanoic ninhydrin). A Daiso IR-60 1002W (40/63 μm) was used for flash column chromatography on silica gel. All reagents and solvents were purchased from either Aldrich Chemical Co., Inc., Kanto Kagaku Co., Inc., Merck & Co., Inc., Nacalai Tesque Company, Ltd., Peptide Institute, Tokyo Chemical Industry Co., Ltd., or FUJIFILM Wako Pure Chemical Corporation, Ltd. and used without further purification unless otherwise indicated. Dichloromethane (CH_2Cl_2) was distilled from phosphorus pentoxide (P_2O_5). Tetrahydrofuran (THF) of anhydrous grade was used. Antibacterial activity test against *M. smegmatis* was performed according to the reported procedure.[12]

*N-Fmoc-MePhe-(2R)-Hiv-OBn (**9**).* To a stirred solution of **12** (0.93 g, 4.45 mmol) in CH_2Cl_2 (25 mL) were added **11** (2.14 g, 5.34 mmol), DMAP (0.65 g, 5.34 mmol), and EDCI·

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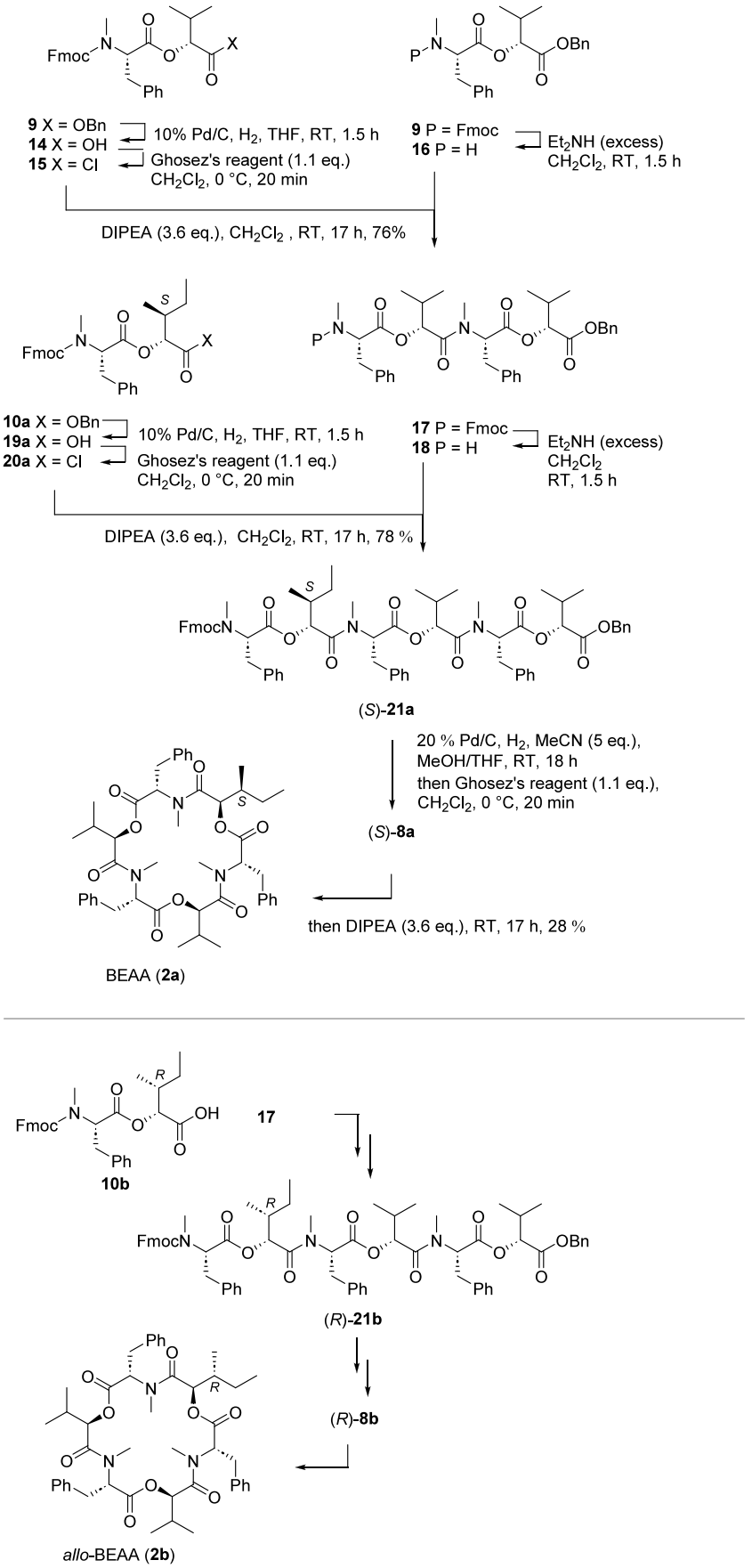
Scheme 3. Total Synthesis of BEAA (2a) and *allo*-BEAA (2b)



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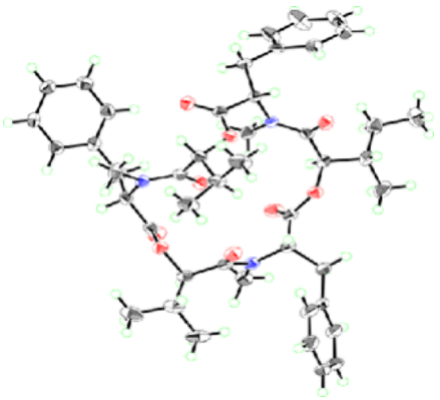
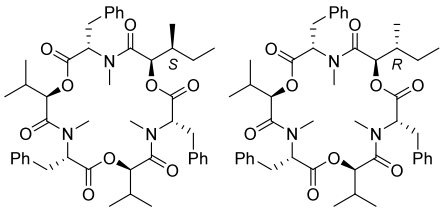


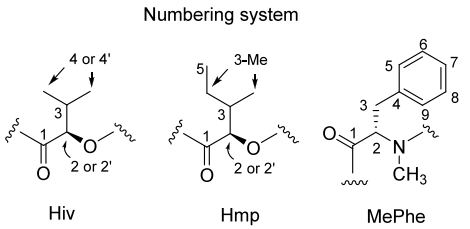
Figure 2. ORTEP structure of synthetic BEAA (**2a**). Supplementary crystallographic data for the structure have been deposited at the Cambridge Crystallographic Data Centre (No. CCDC 2311751).[11]

HCl (1.02 g, 5.34 mmol) at 0 °C under Ar. The mixture was stirred for 14 h at rt and quenched with 1 M aqueous HCl (12 mL) at 0 °C. After separating the organic layers, the water layer was extracted with CH_2Cl_2 (x3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 5:1) to give **9** (2.06 g, 78%) as a colorless viscous oil. $[\alpha]^{24}_{D}$ −44 (*c* 0.60, $CHCl_3$). FTIR (neat) ν_{max} (cm^{-1}) 2966, 1742, 1703, 1452, 1194, 1129, 1022, 741, 699. ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.76 (2H, d, *J* = 7.5 Hz), 7.55−7.32 (9H, m), 7.31−7.17 (6H, m), 7.06 (1H, d, *J* = 6.7 Hz), 5.31−5.21 (1.6H, m), 5.15 (1H, dd, *J* = 12.1, 9.4 Hz), 5.00−4.90 (1H, m), 4.86 (0.4H, d, *J* = 4.0 Hz), 4.39−4.10 (3H, m), 3.43 (0.6H, dd, *J* = 14.7, 5.4 Hz), 3.27 (0.4H, dd, *J* = 14.6, 5.0 Hz), 3.04 (0.6H, dd, *J* = 14.7, 10.9 Hz), 2.90−2.80 (0.4H, m), 2.88 (1.8H, s), 2.86 (1.2H, s), 2.26 (1H, m,), 0.96 (1.8H, d, *J* = 6.9 Hz), 0.90 (4.2H, d, *J* = 6.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 170.8, 170.6, 169.1, 168.9, 156.7, 156.1, 144.0, 143.8, 141.24, 141.20, 136.9, 135.2, 135.1, 128.76, 128.72, 128.56, 128.52, 128.47, 128.41, 127.62, 127.57, 127.03, 127.00, 126.95, 126.8, 126.7, 125.1, 125.0, 124.9, 124.8, 120.0, 119.94, 119.91, 119.87, 77.5, 77.4, 67.8, 67.6, 67.1, 67.0, 59.8, 59.6, 47.2, 47.0, 34.9, 34.7, 31.2, 31.0, 30.1, 30.0, 18.7, 17.04, 16.98. HRMS (ESI) *m*/*z* 614.25189 $[M + Na]^+$ (calcd for $C_{37}H_{37}NO_6Na$, 614.25186).

N-Fmoc-MePhe-(2R,3S)-Hmp-OBn (***10a***). To a stirred solution of **13a** (0.50 g, 2.25 mmol) in CH_2Cl_2 (15 mL) ware added **11** (1.1 g, 2.70 mmol), DMAP (0.30 g, 2.48

Table 1. ^{1}H NMR Data for Natural and Synthetic 2ab ($CDCl_3$, 400 MHz)





	BEAA (**2a**)		*allo*-BEAA (**2b**)	
position	natural[4]	synthetic	biosynthetic[5]	synthetic
Hiv	2 units	2 units	2 units	2 units
2	4.93 (1H, d, 6.2)	4.89 (1H, d, 7.2)	4.86 (2H, d, 8.5)	4.86 (2H, d, 8.5)
	4.96 (1H, d, 6.5)	4.91 (1H, d, 7.2)		
3	2.04 (2H, m)	2.00 (2H, m)	1.98 (2H, m)	1.98 (2H, m)
4	0.43 (3H, d, 6.4)	0.40 (3H, d, 6.5)	0.41 (6H, d, 6.7)	0.41 (6H, d, 6.8)
	0.45 (3H, d, 6.5)	0.42 (3H, d, 6.5)		
4′	0.76 (3H, d, 6.6)	0.77 (3H, d, 6.7)	0.81 (6H, d, 6.6)	0.81 (6H, d, 6.6)
	0.77 (3H, d, 6.8)	0.76 (3H, d, 6.7)		
Hmp	1 unit (2*R*,3*S*)	1 unit (2*R*,3*S*)	1 unit (2*R*,3*R*)	1 unit (2*R*,3*R*)
2	5.04 (1H, d, 7.7)	5.00 (1H, d, 7.8)	4.96 (1H, d, 9.1)	4.97 (1H, d, 9.0)
3	1.78 (1H, m)	1.73 (1H, m)	1.79 (1H, m)	1.79 (1H, m)
4	0.70 (2H, m)	0.68−0.72 (2H, m)	1.37 (1H, m)	1.37 (1H, m)
			0.94 (1H, m)	0.93 (1H, m)
5	0.68 (3H, m)	0.62−0.70 (3H, m)	0.75 (3H, t, 7.4)	0.75 (3H, t, 7.4)
3-Me	0.81 (3H, d, 6.8)	0.80 (3H, d, 6.6)	0.32 (3H, d, 6.8)	0.30 (3H, d, 6.8)
NMePhe	3 units	3 units	3 units	3 units
2	5.43 (3H, m)	5.51 (3H, m)	5.67 (1H, dd, 12, 4.8)	5.65 (1H, dd, 12, 5.0)
			5.51 (2H, m)	5.48 (2H, m)
3	3.35 (3H, m)	3.36 (3H, m)	3.38 (3H, dd, 14.6, 4.8)	3.37 (3H, dd, 14.6, 4.9)
	2.99 (3H, m)	2.89−2.99 (3H, m)	2.95 (3H, m)	2.90−3.00 (3H, m)
5−9	7.22 (15H, m)	7.11−7.30 (15H, m)	7.16−7.28 (15H, m)	7.12−7.31 (15H, m)
N−CH_3	2.95 (3H, s)	2.96 (3H, s)	3.00 (3H, s)	2.99 (3H, s)
	2.99 (6H, s)	3.00 (6H, s)	3.03 (3H, s)	3.00 (3H, s)
			3.05 (3H, s)	3.04 (3H, s)

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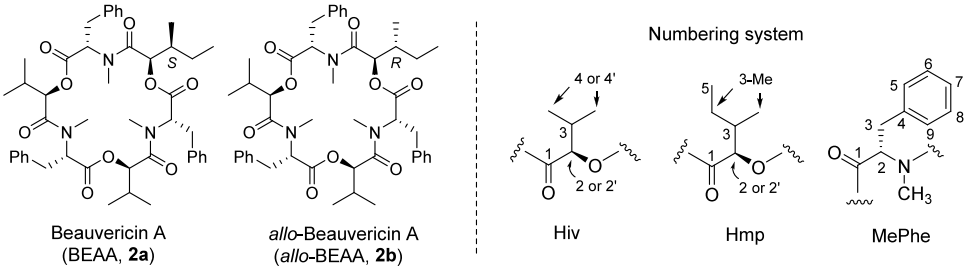
Table 2. ^{13}C NMR Data for 2ab ($CDCl_3$, 101 MHz)



Beauvericin A (BEAA, **2a**) | *allo*-Beauvericin A (*allo*-BEAA, **2b**)

	BEAA (**2a**)		*allo*-BEAA (**2b**)	
position	natural[4]	synthetic	biosynthetic[5]	synthetic
Hiv	2 units	2 units	2 units	2 units
1 C=O	169.94 or 169.24	169.96 or 169.94 or 169.91 or 169.48 or 169.44	169.78, 169.64	169.64
2	75.4	75.52, 75.48	75.64	75.63
3	29.7	29.71, 29.69	29.74, 29.70	29.75, 29.72
4	17.54	17.46, 17.43	17.43	17.48, 17.43
4′	18.29	18.28, 18.26	18.32	18.35, 18.34
Hmp	1 unit (2*R*,3*S*)	1 unit (2*R*,3*S*)	1 unit (2*R*,3*R*)	1 unit (2*R*,3*R*)
1 C=O	169.94 or 169.24	169.96 or 169.94 or 169.91 or 169.48 or 169.44	169.61	169.92
2	74.22	74.30	74.27	74.25
3	35.81	35.84	35.62	35.62
4	24.47	24.40	24.53	24.55
5	11.31	11.30	10.63	10.63
3-Me	14.34	14.35	13.27	13.27
NMePhe	3 units	3 units	3 units	3 units
1 C=O	169.94 or 169.24	169.96 or 169.94 or 169.91 or 169.48 or 169.44	169.99, 169.94, 169.92	169.97, 169.92, 169.89
2	57.53, 57.52	57.30, 57.16	57.36, 56.64	57.35, 56.64
3	34.83, 34.68	34.79, 34.68, 34.62	34.84, 34.73, 34.62	34.87, 34.75, 34.64
4	136.64	136.60, 136.59, 136.56	136.64, 136.58, 136.48	136.66, 136.60, 136.49
5–9	128.92, 128.53, 126.76	128.86, 128.83, 128.82, 128.51, 128.45, 126.75	128.81, 126.77, 128.53	128.84, 128.82, 128.80, 128.53, 126.78
N–CH_3	32.43, 32.37	32.22, 32.19	32.33, 31.86	32.35, 31.87

mmol), and EDCI·HCl (0.42 g, 2.70 mmol) at 0 °C under Ar. The mixture was stirred for 14 h at rt, quenched with 1 M aqueous HCl (12 mL) at 0 °C. After separating the organic layers, the water layer was extracted with CH_2Cl_2 (x3). The combined organic layers were washed with the saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 5:1) to give **10a** (0.99 g, 75%) as a colorless viscous oil. $[\alpha]^{24}_D$ −37 (*c* 1.2, $CHCl_3$). FTIR (neat) ν_{max} (cm^{-1}) 2964, 1742, 1704, 1452, 1196, 1132, 741, 699. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.4 Hz), 7.53−7.16 (15H, m), 7.07 (1H, d, *J* = 6.6 Hz), 5.30−5.10 (3.2H, m), 5.04 (0.4H, d, *J* = 3.2 Hz), 4.97 (0.4H, dd, *J* = 10.8, 5.0 Hz), 4.37−4.15 (2.6H, m), 4.11 (0.4H, t, *J* = 6.5 Hz), 3.41 (0.6H, dd, *J* = 14.7, 5.4 Hz), 3.27 (0.4H, dd, *J* = 14.6, 5.0 Hz), 3.03 (0.6H, dd, *J* = 14.7, 11.0 Hz), 2.93−2.82 (0.4H, m), 2.87 (3H, s), 2.01 (1H, m), 1.43−1.14 (2H, m), 0.90−0.80 (6H, m). ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 170.8, 170.6, 169.4, 169.2, 156.7, 156.1, 144.01, 143.97, 143.9, 143.8, 141.24, 141.20, 136.9, 135.2, 135.1, 128.8, 128.7, 128.61, 128.58, 128.53, 128.49, 128.45, 128.4, 127.63, 127.58, 127.04, 127.00, 126.96, 126.8, 126.7, 125.2, 125.0, 124.9, 124.8, 119.94, 119.91, 119.88, 75.6, 75.5, 67.8, 67.7, 67.14, 67.07, 59.9, 59.6, 47.2, 47.0, 36.5, 36.4, 34.9, 34.7, 31.1, 31.0, 26.0, 14.3, 14.2, 11.6. HRMS (ESI) *m*/*z* 628.26755 $[M + Na]^+$ (calcd for $C_{38}H_{39}NO_6Na$, 628.26751).

N-Fmoc-MePhe-(2R,3R)-Hmp-OBn (***10b***). To a stirred solution of **13b** (0.22 g, 0.967 mmol) in CH_2Cl_2 (6.0 mL) were added **11** (0.54 g, 1.35 mmol), DMAP (0.15 g, 1.26 mmol), and EDCI·HCl (0.26 g, 1.35 mmol) at 0 °C under Ar. The mixture was stirred for 14 h at rt, quenched with 1 M aqueous HCl (3.0 mL) at 0 °C. After separating the organic layers, the water layer was extracted with CH_2Cl_2 (x3). The combined organic layers were washed with the saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 5:1) to give **10b** (0.41 g, 70%) as a colorless viscous oil. $[\alpha]^{24}_D$ −40 (*c* 0.80, $CHCl_3$). FTIR (neat) ν_{max} (cm^{-1}) 2964, 1743, 1703, 1452, 1309, 1193, 741, 699. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (2H, d, *J* = 7.4 Hz), 7.53−7.18 (15H, m), 7.05 (1H, d, *J* = 6.6 Hz), 5.30−4.88 (4H, m), 4.37−4.04 (3H, m), 3.41 (0.6H, dd, *J* = 14.7, 5.4 Hz), 3.25 (0.4H, dd, *J* = 14.6, 5.1 Hz), 3.03 (0.6H, dd, *J* = 14.7, 10.9 Hz), 2.92−2.77 (0.4H, m), 2.87 (1.8H, s), 2.84 (1.2H, s), 2.00 (1H, m), 1.38 (1H, m), 1.20 (1H, m), 0.95−0.75 (6H, m). ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 170.8, 170.6, 169.2, 168.9, 156.7, 156.1, 144.0, 143.9, 143.8, 141.25, 141.21, 136.9, 135.2, 135.1, 128.8, 128.7, 128.60, 128.57, 128.53, 128.48, 128.4, 127.63, 127.59, 127.04, 127.00, 126.96, 126.8, 126.7, 125.1, 125.0, 124.9, 124.8, 120.0, 119.92, 119.88, 67.8, 67.6, 67.1, 67.0, 59.8, 59.6, 47.2, 47.1, 36.5, 34.9, 34.7, 31.2, 31.0, 24.5, 24.4, 15.4, 11.5, 11.4. HRMS

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(ESI) m/z 628.26751 $[M + Na]^+$ (calcd for $C_{38}H_{39}NO_6Na$, 628.26751).

*N-Fmoc-MePhe-(2R)-Hiv-MePhe-(2R)-Hiv-OBn (**17**).* To a stirred solution of **9** (250 mg, 0.423 mmol) in THF (0.5 mL) was added Et_2NH (1.0 mL) at rt. The mixture was stirred for 1.5 h and concentrated under reduced pressure. The crude amine **16** was used for the next condensation step without further purification.

To a mixture of Pd/C (50.0 mg, 10% on charcoal) in THF (1.5 mL) was added a solution of **9** (250 mg, 0.423 mmol) in THF (3.0 mL). The suspension was stirred for 1.5 h at rt under H_2 (balloon) and filtered through a thin Celite pad. The filtrate was concentrated under reduced pressure. The crude acid **14** was used for the next step without further purification. To a stirred solution of the crude acid **14** in CH_2Cl_2 (1.0 mL) was added Ghosez's reagent (152 μL, 1.16 mmol) at 0 °C. The mixture was stirred for 20 min at the same temperature to form acid chloride **15** *in situ*. This acid chloride **15** was used for the next condensation step without further purification. A stirred solution of crude amine **16** in CH_2Cl_2 (1.0 mL) was added to a solution of the acid chloride **15** in CH_2Cl_2 (1.0 mL) at 0 °C. The mixture was stirred for 20 min at the same temperature, and then *N,N*-diisopropylethylamine (258 μL, 1.52 mmol) was added to the mixture at 0 °C. The mixture was stirred for 17 h at rt, quenched with H_2O, and extracted with CH_2Cl_2 (x3). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 4:1) to give **17** (275 mg, 76%) as a viscous oil. $[\alpha]^{24}_D$ −47 (*c* 1.9, $CHCl_3$). FTIR (neat) ν_{max} (cm^{-1}) 2966, 1739, 1704, 1666, 1453, 1195, 1025, 741, 698. ^1H NMR (400 MHz, $CDCl_3$) δ 7.77−7.70 (2H, m), 7.55−7.05 (21H, m), 5.69 (1H, dd, J = 12.1, 4.7 Hz), 5.26 (1H, d, J = 12.1 Hz), 5.19 (0.6H, J = 11.1, 5.1 Hz), 5.14 (1H, d, J = 12.1 Hz), 5.04−4.88 (2.4H, m), 4.33−4.05 (3H, m), 3.47 (1H, dd, J = 14.9, 4.7 Hz), 3.39 (0.6H, dd, J = 14.6, 5.2 Hz), 3.27 (0.4H, dd, J = 14.6, 4.7 Hz), 3.08−2.84 (2H, m), 2.94−2.90 (6H, m), 2.30 (1H, m), 1.56 (1H, m), 1.07−0.95 (6H, m), 0.72−0.60 (3H, m), 0.55−0.45 (3H, m). ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 170.6, 170.4, 170.3, 169.9, 169.63, 169.60, 169.30, 169.27, 156.7, 156.2, 144.1, 144.0, 143.9, 143.8, 141.2, 137.1, 136.61, 136.58, 135.2, 128.7, 128.63, 128.59, 128.53, 128.50, 128.47, 127.6, 127.5, 127.04, 127.00, 126.96, 126.85, 126.82, 126.7, 126.6, 125.2, 125.1, 125.0, 124.9, 119.93, 119.88, 119.85, 77.8, 75.5, 75.3, 67.7, 67.6, 67.1, 59.9, 59.7, 57.3, 47.2, 47.0, 34.9, 34.7, 34.6, 31.7, 31.3, 31.1, 30.1, 29.4, 29.3, 19.04, 18.95, 18.61, 18.55, 17.0, 16.9, 16.5, 16.3. HRMS (ESI) m/z 875.38846 $[M + Na]^+$ (calcd for $C_{52}H_{56}N_2O_9Na$, 875.38835).

*N-Fmoc-MePhe-(2R,3S)-Hmp-NMePhe-(2R)-Hiv-MePhe-(2R)-Hiv-OBn (**21a**).* To a stirred solution of **17** (41.0 mg, 47.8 μmol) in THF (0.5 mL) was added Et_2NH (1.0 mL) at rt. The mixture was stirred for 1.5 h and concentrated under reduced pressure. The crude amine **18** was used for the next condensation step without further purification. To a mixture of Pd/C (6.0 mg, 10% on charcoal) in THF (0.2 mL) was added a solution of **10a** (32.0 mg, 0.0528 mmol) in THF (0.5 mL). The suspension was stirred for 1.5 h at rt under H_2 (balloon) and filtered through a thin Celite pad. The filtrate was concentrated under reduced pressure. The crude acid **19a** was used for the next step without further purification. To a stirred solution of the crude acid **19a** in CH_2Cl_2 (0.5 mL) was added Ghosez's reagent (17 μL, 0.131 mmol) at 0 °C. The mixture was stirred for 20 min at the same temperature to form acid chloride **20a** *in situ*. This acid chloride **20a** was used for the next condensation step without further purification.

A stirred solution of crude amine **18** in CH_2Cl_2 (0.5 mL) was added to a solution of the acid chloride **20a** in CH_2Cl_2 (0.5 mL) at 0 °C. The mixture was stirred for 20 min at the same temperature, and then *N,N*-diisopropylethylamine (29 μL, 0.172 mmol) was added to the mixture at 0 °C. The mixture was stirred for 17 h at rt, quenched with H_2O, and extracted with CH_2Cl_2 (x3). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude residue was roughly purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 3:1) to give **21a** (42.0 mg, 48%). $[\alpha]^{24}_D$ −44 (*c* 2.0, $CHCl_3$). FTIR (neat) ν_{max} (cm^{-1}) 3727, 3706, 3627, 3599, 2965, 1739, 1704, 1666, 1455, 1198, 1021, 740, 698. ^1H NMR (400 MHz, $CDCl_3$) *d* 7.80−7.70 (2H, m), 7.55−7.10 (26H, m), 5.80−5.55 (2H, m), 5.30−5.10 (3H, m), 5.09−4.90 (2H, m), 4.45−4.00 (3H, m), 3.50−3.20 (3H, m), 3.10−2.80 (11H, m), 2.04 (1H, m), 1.50 (2H, m), 1.40−1.20 (2H, m), 0.98 (3H, d, J = 6.8 Hz), 0.95−0.80 (6H, m), 0.76 (2H, d, J = 6.9 Hz), 0.72 (1H, d, J = 6.9 Hz), 0.66 (2H, d, J = 6.9 Hz), 0.63 (1H, d, J = 6.9 Hz), 0.54 (3H, d, J = 6.8 Hz), 0.51−0.43 (3H, m). ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 170.6, 170.3, 170.2, 170.1, 170.0, 169.8, 169.6, 156.7, 144.0, 143.92, 143.86, 143.8, 141.19, 141.17, 139.6, 137.2, 136.9, 136.7, 136.64, 136.58, 135.2, 129.3, 128.8, 128.73, 128.66, 128.63, 128.61, 128.55, 128.51, 128.44, 128.39, 127.9, 127.81, 127.76, 127.6, 127.5, 127.0, 126.9, 126.8, 126.75, 126.6, 125.2, 125.1, 125.0, 124.5, 124.32, 120.28, 120.04, 120.01, 119.91, 119.87, 119.8, 75.9, 75.8, 75.6, 75.3, 71.1, 67.7, 67.6, 67.28, 67.26, 67.14, 67.1, 59.9, 59.63, 59.59, 59.55, 57.32, 57.25, 48.6, 47.1, 47.0, 36.5, 36.4, 34.9, 34.8, 34.7, 34.6, 32.0, 31.9, 31.6, 31.4, 31.2, 31.1, 29.4, 29.3, 29.2, 25.92, 25.89, 25.7, 19.1, 19.0, 18.9, 16.4, 16.2, 15.99, 15.95, 15.9, 14.23, 14.2, 14.1, 13.9, 13.3, 11.7, 11.6. HRMS (ESI) m/z 1150.54046 $[M + Na]^+$ (calcd for $C_{68}H_{77}N_3O_{12}Na$, 1150.54049).

*N-Fmoc-MePhe-(2R,3R)-Hmp-MePhe-(2R)-Hiv-MePhe-(2R)-Hiv-OBn (**21b**).* To a stirred solution of **17** (53.0 mg, 62.1 μmol) in THF (0.5 mL) was added Et_2NH (1.0 mL) at rt. The mixture was stirred for 1.5 h at the same temperature and concentrated under reduced pressure. The crude amine **18** was used for the next condensation step without further purification.

To a mixture of Pd/C (10.0 mg, 10% on charcoal) in THF (1.0 mL) was added a solution of **10b** (54.0 mg, 89.1 μmol) in THF (1.0 mL). The suspension was stirred for 1.5 h at rt under H_2 (balloon) and filtered through a thin Celite pad. The filtrate was concentrated under reduced pressure. The crude acid **19b** was used for the next step without further purification. To a stirred solution of the crude acid **19b** in CH_2Cl_2 (0.5 mL) was added Ghosez's reagent (22 μL, 167 μmol) at 0 °C. The mixture was stirred for 20 min at the same temperature to form acid chloride **20b** *in situ*. This acid chloride **20b** was used for the next condensation step without further purification.

A stirred solution of crude amine **18** in CH_2Cl_2 (0.5 mL) was added to a solution of the acid chloride **20b** in CH_2Cl_2 (0.5 mL) at 0 °C. The mixture was stirred for 20 min at the same temperature, and then *N,N*-diisopropylethylamine (38 μL, 224 μmol) was added to the mixture at 0 °C. The mixture was stirred for 17 h at rt, quenched with H_2O, and extracted

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with CH_2Cl_2 (x2). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under a reduced pressure. The crude residue was purified by silica gel flash column chromatography (*n*-hexane:EtOAc = 3:1) to give **21b** (53.0 mg, 75%) as a viscous oil. $[\alpha]^{24}_D$ −48 (*c* 1.2, $CHCl_3$). FTIR (neat): ν_{max} (cm^{-1}) 2965, 1738, 1704, 1665, 1453, 1021, 741, 699. ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (2H, t, J = 7.7 Hz), 7.55−7.05 (26H, m), 5.64 (2H, td, J = 12.9, 4.5 Hz), 5.26 (1H, d, J = 12.1 Hz), 5.19 (1H, m), 5.14 (1H, d, J = 12.1 Hz), 4.35−4.00 (3H, m), 3.55−3.20 (3H, m), 3.10−2.90 (3H, m), 3.03 (3H, s), 2.91 (6H, s), 2.06 (1H, m), 1.54 (2H, m), 1.42 (2H, m), 0.98 (3H, d, J = 6.9 Hz), 0.89 (3H, t, J = 7.4 Hz), 0.76 (1.8H, d, J = 6.9 Hz), 0.72 (1.2H, d, J = 6.9 Hz), 0.66 (1.8H, d, J = 6.9 Hz), 0.63 (1.2H, d, J = 6.9 Hz), 0.55 (3H, d, J = 6.7 Hz), 0.47 (3H, dd, J = 9.0, 6.7 Hz). ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 170.6, 170.3, 170.2, 170.13, 170.11, 170.05, 169.8, 169.6, 169.3, 156.7, 156.2, 144.1, 143.94, 143.85, 141.2, 137.2, 136.9, 136.8, 136.6, 135.2, 128.8, 128.74, 128.68, 128.62, 128.57, 128.54, 128.49, 128.45, 127.5, 127.11, 127.06, 127.0, 126.96, 126.9, 126.8, 126.7, 126.6, 125.2, 125.1, 125.0, 124.9, 119.92, 119.88, 119.8, 75.8, 75.5, 75.3, 67.73, 67.66, 67.1, 59.9, 59.6, 57.4, 57.3, 47.2, 47.1, 36.6, 34.9, 34.8, 34.7, 34.6, 32.02, 31.97, 31.7, 31.2, 31.1, 29.4, 29.3, 29.2, 24.1, 19.1, 19.0, 18.9, 16.4, 16.3, 16.1, 16.0, 15.3, 11.7. HRMS (ESI) m/z 1150.54061 $[M + Na]^+$ (calcd for $C_{68}H_{77}N_3O_{12}Na$, 1150.54049).

*Beauvericin A (BEAA, **2a**).* To a suspension of Pd/C (26 mg, 10% on charcoal) in MeOH/THF (0.8 mL, 1:1 v/v) were added a solution of **21a** (88.0 mg, 0.0783 mmol) in MeOH/THF (0.8 mL, 1:1) and MeCN (20 μL, 0.392 mmol) under H_2. The mixture was stirred for 18 h at rt under H_2 (balloon) and filtered through a thin Celite pad. The Celite pad was washed with EtOAc. The filtrate was concentrated under reduced pressure. The acid chloride **8a** was employed for the cyclization reaction without purification.

To a solution of the acid chloride **8a** in CH_2Cl_2 (12 mL) was added Ghosez's reagent (28 μL, 0.215 mmol) at 0 °C. The mixture was stirred for 20 min at 0 °C, and then *N,N*-diisopropylethylamine (36 μL, 0.282 mmol) was added at room temperature. The mixture was stirred for 17 h at rt, diluted with water, and extracted with CH_2Cl_2 (x2). The combined organic layers were washed with brine, dried in $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by high-performance C18 reversed phase silica gel column chromatography (5C18-AR-2, Nacalai tesque, Inc., H_2O/MeCN = 3:7) to give **2a** as a white powder (17.2 mg, 28%). $[\alpha]^{25}_D$ +62 (*c* 1.13, $CHCl_3$, $[\alpha]^{25}_D$ +49 (*c* 0.50, MeOH) [lit. Five $[\alpha]^{25}_D$ +57 (*c* 0.18, MeOH)]. mp 156−157 °C. FTIR (neat): ν_{max} (cm^{-1}) 2964, 1743, 1662, 1180, 747, 699. HRMS (ESI) m/z 820.41545 $[M + Na]^+$ (calcd for $C_{46}H_{59}N_3O_9Na$, 820.41490).

*allo-Beauvericin A (allo-BEAA, **2b**).* To a suspension of Pd/C (26 mg, 10% on charcoal) in MeOH/THF (0.8 mL, 1:1) were added a solution of **21b** (40 mg, 0.0354 mmol) in MeOH/THF (0.8 mL, 1:1) and MeCN (9.4 μL, 0.180 mmol) under H_2. The mixture was stirred for 18 h at rt under H_2 (balloon) and filtered through a thin Celite pad. The Celite pad was washed with EtOAc. The filtrate was concentrated under reduced pressure. The acid chloride **8b** was employed for the cyclization reaction without purification.

To a solution of the acid chloride **8b** in CH_2Cl_2 (6.0 mL) was added Ghosez's reagent (13 μL, 0.0974 mmol) at 0 °C. The mixture was stirred for 20 min at 0 °C, and then *N,N*-diisopropylethylamine (20 μL, 0.127 mmol) was added at room temperature. The mixture was stirred for 17 h at rt, diluted with water, and extracted with CH_2Cl_2 (x2). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by high-performance C18 reversed phase silica gel column chromatography (5C18-AR-2, Nacalai Tesque, Inc., H_2O/MeCN = 3:7) to give **2b** as a white powder (5.9 mg, 21%). $[\alpha]^{25}_D$ +60 (*c* 0.64, $CHCl_3$), $[\alpha]^{25}_D$ +41 (*c* 0.50, MeOH) [lit. Five $[\alpha]^{25}_D$ +60 (*c* 0.20, MeOH)]. mp 159−160 °C. FTIR (neat): ν_{max} (cm^{-1}) 2964, 1747, 1663, 1182, 1017, 744, 699. HRMS (ESI) m/z 820.41467 $[M + Na]^+$ (calcd for $C_{46}H_{59}N_3O_9Na$, 820.41490).

*X-Ray Crystallographic Analysis of BEAA (**2a**).* A colorless block crystal of **2a** from hexanes at room temperature was mounted on the CryoLoop with Palaton oil and placed in the N_2 stream at 110 K. Unit cell dimensions and data reduction were done by using the CrysAlisPro software package (Rigaku Oxford Diffraction, 2020). Absorption corrections were applied using the Multi Scan method. The structures were solved using direct methods (SHELXS-97, Sheldrick GM. University of Gottingen; Germany:1997) and refined by full-matrix least-squares on F^2 using SHELXL-2018/3 (Sheldrick GM. University of Gottingen; Germany: 2018). The X-ray data have been deposited at the Cambridge Crystallographic Data Center (CCDC 2311751).[11]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00278.

Experimental details and copies of ^1H and ^{13}C NMR spectra (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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