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Synthesis of Microcin SF608

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The first total synthesis of aquatic peptide microcin SF608 is described. Coupling of L-Hpla with the dipeptide L-Phe-L-Choi followed by coupling with agmatine and a deprotection step gave microcin SF608. In addition, the levorotatory character of L-Hpla (**5**) was thoroughly established, and the conformational analysis of L-Choi containing peptides **1** and **8**−**10** was performed using NMR spectroscopy to examine the cis−trans isomer equilibrium of the L-Phe-L-Choi amide bond.

Introduction

Many peptides of aquatic origin involving unusual amino acids have interesting biological activities as well as unique structures.[1] Microcin SF608 was isolated in Israel from a nontoxic strain of the cyanobacterium *Microcystis aeruginosa* and inhibited trypsin with IC_{50} of 0.5 μg/mL, but not chymotrypsin and neprolysine at 20.0 μg/mL.[2] The peptide microcin SF608 is made up of the two amino acids L-phenylalanine (L-Phe) and the unusual 2-carboxy-6-hydroxyoctahydroindole (Choi), the α-hydroxy acid *p*-hydroxyphenyllactic acid (L-Hpla), and agmatine (Agma), the decarboxylated arginine (Figure 1). This tetraunit structure resembles that of aeruginosins, a group of aquatic peptides isolated in Japan[3] for which we have recently established a synthetic entry, having achieved the first total synthesis and reassigned the structure of aeruginosins 298.[4,5] Interestingly, microcin SF608 (**1**) has the L configuration in the α-amino acid attached to the nitrogen of the Choi unit, while aeruginosins incorporate a D-amino acid at this point.[4b,6] Moreover, the Hpla fragment in **1** is also of the L configuration, in contrast to the D-Hpla derivatives found in aeruginosins.

In this paper we describe the first total synthesis of microcin SF608 that serves to confirm the structural assignment of **1** and establishes its absolute configuration.

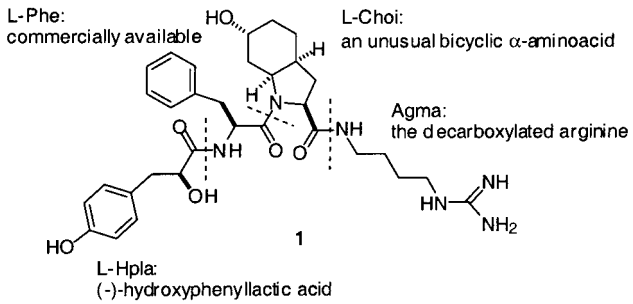

FIGURE 1. Subunits of the aquatic peptide microcin SF608.

Results and Discussion

A Preliminary Question: The Hydroxyphenyllactic Acid (Hpla). As a prelude to the requisite coupling reactions for the synthesis of microcin SF608, we needed the corresponding four subunits (Figure 1). The preparation of L-Hpla merits some comments, since there is some confusion in the literature about the correlation of the nature (*d* or *l*) of the rotatory power with the absolute configuration of L-Hpla. The diprotected form of the L-isomer of *p*-hydroxyphenyllactic acid [AcO-L-Hpla(Bn)-OH] (**2**), required for the synthetic plan, was synthesized from *O*-benzyl-L-tyrosine (Scheme 1) through a diazotation process, using isoamyl nitrite in AcOH medium,[7] which allows the formation of the protected form of L-Hpla in only one step. The ee of L-Hpla, which was 70% after the acetoxylation process, was increased up to >98% after a crystallization process with (−)-1-phenylethylamine. The retention of the *S* configuration in **2**, due to the participation of the neighboring carboxyl group in the substitution process, was additionally confirmed by converting **2** into the methoxyphenylacetic (MPA) ester derivative **3**[8] and deducing the absolute configuration of the secondary alcohol from its ^{1}H NMR spectra recorded at different temperatures according to

(1) For an overview: Shioiri, T.; Hamada, Y. *Synlett* **2001**, 184−201.

(2) (a) Banker, R.; Carmeli, S.*Tetrahedron* **1999**, *55*, 10835−10844. (b) For a new isolation, see: Reshef, V.; Carmeli, S. *Tetrahedron* **2001**, *57*, 2885−2894.

(3) Ishida, K.; Okita, H.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron*. **1999**, *55*, 10971−10988.

(4) (a) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc.* **2000**, *122*, 11248−11249. (b) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *Chem. Eur. J.* **2001**, *7*, 3446−3460.

(5) For another synthesis of aeruginosin 298-A, see: Wipf, P.; Methot, J.-L.*Org. Lett.* **2000**, *2*, 4213−4216.

(6) For the influence of phenylalanine configuration on Phe-Ψ-Pro thrombin inhibitors, see Wagner, J.; Kallen, J.; Ehrhardt, C.; Evenou, J.-P.; Wagner, D. *J. Med. Chem.* **1998**, *41*, 3664−3674.

(7) For the use of isoamyl nitrite in acetic medium for the formation of α-acetoxy acids from α-amino acids, see: Schmidt, U.; Kroner, M.; Griesser, H. *Synthesis* **1989**, 832−835.

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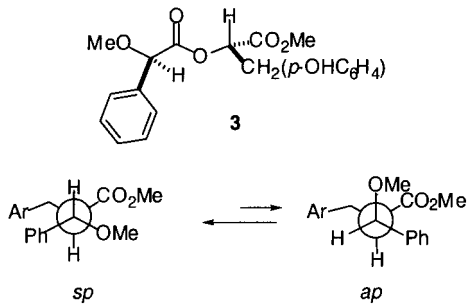
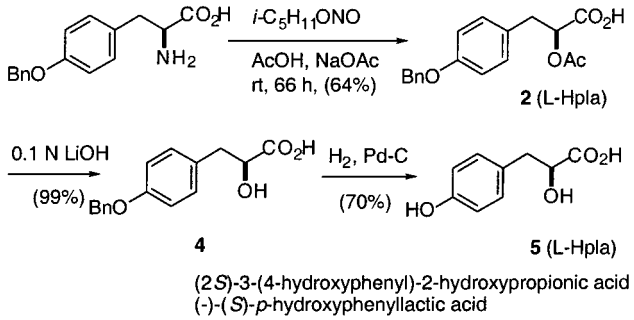


FIGURE 2. *sp* and *ap* conformations corresponding to the (*R*)-MPA ester 3.

SCHEME 1. Synthesis of L-Hpla



Riguera's method.[9] The simplified Riguera protocol relies on the thermodynamic preference for the *sp* conformer rather than the *ap* conformation associated with MPA esters (Figure 2). At low temperatures the population of the *sp* conformer is increased, resulting in either a downfield shift [0.07 ppm for the methyl protons of the methoxycarbonyl group in **3** not shielded by the aryl ring of (*R*)-MPA] or an upfield shift (0.04 ppm for the shielded aromatic protons of Hpla in **3**) relative to the observed shift of the same protons at room temperature.

Having ensured the absolute configuration of **2**, we converted it into L-Hpla, (*S*)-2-hydroxy-3-(4-hydroxyphenyl)propionic acid (**5**) (two steps: saponification and hydrogenolytic cleavage), enabling us to conclude that L-Hpla is the levorotatory enantiomer. This result allows us to clarify some mistakes that have appeared in the literature;[10,11] it now being clear that the structure of natural products isolated from *Pterocarpus Marsupium*[10b] and *Aristolochia kaemferi*[10c] should be revised and their absolute configuration reassigned as (2*S*)-3-(*p*-hydroxyphenyl)lactic acid and sodium (2*S*)-(*p*-hydroxyphenyl)-lactate, respectively. Moreover the correlation of the absolute configuration of L-Hpla with its levorotatory character affords a complementary procedure to that of the *l*-menthol method for the structural elucidation of Hpla-containing natural products.[12−14]

Synthesis of Microcin SF608. To assemble the structure of microcin SF608, having prepared Hpla derivative **2**, we needed the other subunits with the adequate protecting groups for a peptide synthesis. The protected agmatine **6** was prepared by guanidinylation of 1,4-diaminobutane with *N*,*N*′-di-*tert*-butoxycarbonyl thiourea following the protocol described in the literature.[15] The synthesis of *N*-Boc-L-Choi(OMe) (**7**) was carried out in six steps starting from *O*-methyl-L-tyrosine according to our recently described methodology.[4b] After obtaining the four units L-Hpla, L-Phe, L-Choi, and Agma in an appropriate protected form, the synthesis of microcin SF608 was carried out as outlined in Scheme 2.

Removal of the Boc group from L-Choi **7** followed by coupling to Boc-Phe-OH, using PyBOP in the presence of NMM, gave dipeptide **8** as a separable mixture of alcohol **8a** and its trifluoroacetate **8b** in 90% combined yield. Boc group removal from **8** followed by coupling with L-Hpla in the same reaction conditions gave peptide **9**. Although peptides **8** and **9** were isolated as a mixture of compounds with both free and trifluoroacetylated Choi hydroxyl groups, it did not constitute a synthetic problem, and the analytical process was only slightly longer since both compound-types can be easily separated.

Saponification of the methyl ester in **9**, which induces concomitant deprotection of the secondary alcohol hydroxyl groups, followed by coupling with di-Boc-agmatine (**6**) furnished **10**. Cleavage of the orthogonal protecting groups in two high-yielding steps afforded the target compound **1**. The synthetic microcin SF608 coeluted with authentic material (supplied by Prof. Carmeli) by reversed phase analytical HPLC (60:40 H_2O/CH_3CN; 0.05% TFA), and the ^1H and ^{13}C NMR spectra of the samples in DMSO were identical,[2a] although a slight reassignment should be carried out.[16]

(8) L-Hpla (**2**) was esterified at room temperature (MeOH/HCl) in quantitative yield. The resulting hydroxy ester was coupled with (*R*)-(−)-2-phenyl-2-methoxyacetic acid in THF using HOBt, pyridine and DCC, according to the experimental procedure reported in ref 9. The purified compound **3** (Florisil, hexane/EtOAc 2:1) showed the following ^1H NMR data (300 MHz, $CDCl_3$): 2.96 (dd, J = 13.4, 8.4 Hz, 1H), 3.50 (dd, J = 14, 4.4 Hz, 1H), 3.379 (s, 3H), 3.71 (s, 3H), 4.83 (s, 1H), 5.01 (s, 2H), 5.21 (dd, J = 8.5, 4 Hz, 1H), 6.73 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 7.30−7.42 (m, 10H); (300 MHz, $CDCl_3$) (−50 °C): 3.01 (m, 2H), 3.386 (s, 3H), 3.77 (s, 3H), 4.90 (s, 1H), 5.00 (s, 2H), 5.20 (dd, J = 7.9, 4.6 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 7.35−7.47 (m, 10H).

(9) Latypov, S. K.; Seco, J.-M.; Quinoá, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 877−882.

(10) (a) Maurya[10b] and Wu[10c] wrongly reported that the configuration of the levorotatory isomer of 3-*p*-hydroxyphenyllactic was 2*R*, and Hattori[10d] erroneously claimed that the dextrorotatory isomer was 2*S*. (b) Maurya, R.; Ray, A. B.; Duah, F. K.; Slatkin, D.-J.; Schiff, P. J., Jr. *J. Nat. Prod.* **1984**, *47*, 179−181. (c) Wu, T.-S.; Leu, Y.-L.: Chan, Y.-Y. *Chem. Pharm. Bull.* **1998**, *46*, 6, 1624−1626. (d) Hattori, K.; Takahashi, K. *Supramol. Chem.* **1993**, *2*, 209−213.

(11) (a) The dextrorotatory isomer of *p*-hydroxyphenyllactic acid is of course the *R* isomer, which agrees with the results reported by Satake[11b] and ourselves.[4] Unfortunately, the prestigious Beilstein Institut has wrongly introduced Satake's data, by including it in the section corresponding to the *S* isomer. (b) Satake, T.; Kamiya, K.; Saiki, Y.; Hama, T.; Fujimoto, Y.; Kitanaka, S.; Kimura, Y.; Uzawa, J.; Endang, H.; Umar M. *Chem. Pharm. Bull.* **1999**, *47*, 1444−1447.

(12) (a) The *l*-menthol method[12b] (retention time of menthyl esters of Hpla in HPLC) has been used to determine the absolute configuration of the Hpla moiety in several structural elucidation of natural products. (b) Tsukamoto, S.; Painuly, P.; Young, K. A.; Yang, X.; Shimizu, Y.; Cornell, L. *J. Am. Chem. Soc.* **1993**, *115*, 11046−11047.

(13) Murakami has described the synthesis of D- and L-Hpla from the corresponding *p*-aminophenylanalines with nearly 30% yield in each case.[3,14] Their absolute configuration was established by the Shimizu method,[12] and there are no comments about the rotatory power of these enantiomers.

(14) Matsuda, H.; Okino, T.; Murakami, M.; Yamaguchi, K. *Tetrahedron* **1996**, *52*, 14501−14506.

(15) (a) Expósito, A.; Fernández-Suárez, M.; Iglesias, T.; Muñoz, L.; Riguera, R. *J. Org. Chem.* **2001**, *66*, 4206−4213. (b) Iwanovicz, E. J.; Poss, M. A.; Lin, J. *Synth. Commun.* **1993**, *23*, 1443−1445.

(16) The NMR data reported for the methylene carbons at C-4 and C-5 of the Choi nucleus in the isolation of microcin SF608[2a] should be reassigned: H-4 (δ 1.40 and 2.03 for the trans rotamer and δ 1.32 and 1.92 for the cis rotamer, all of them as multiplets) and H-5 (δ 1.45 for both protons in each rotamer as a multiplet signal); C-4 (δ 19.1) and C-5 (δ 26.1) in both rotamers.

SCHEME 2. Synthesis of Microcin SF608 (1)

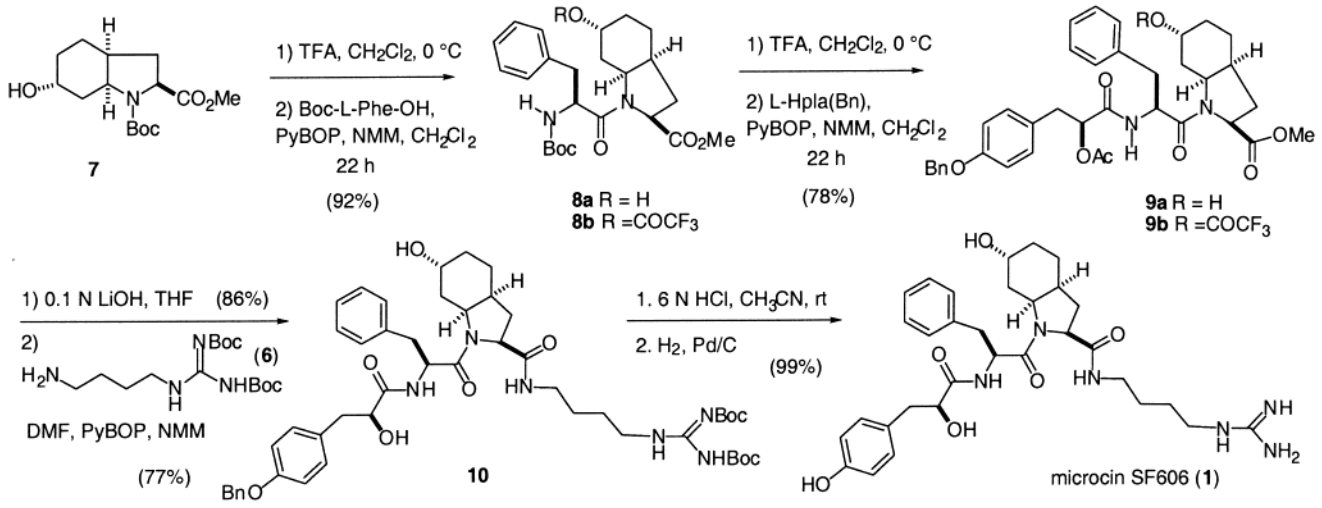


TABLE 1. Proton Chemical Shifts Values of L-Choi Derivatives (trans-cis Rotamers). Comparison between Microcin SF608 Series and Aeruginosins 298

	H-2		H-7a		
compound	trans	cis	trans	cis	peptide type
1	4.23	3.90	4.41	4.09	L-Phe-L-Choi
8a	4.42	3.34	4.42	4.30	L-Phe-L-Choi
9a	4.26	3.68	4.42	4.14	L-Phe-L-Choi
298-A[4b]	4.17	4.63	4.04	4.28	D-Leu-L-Choi
298-B[4b]	4.12	4.64	4.02	4.27	D-Leu-L-Choi

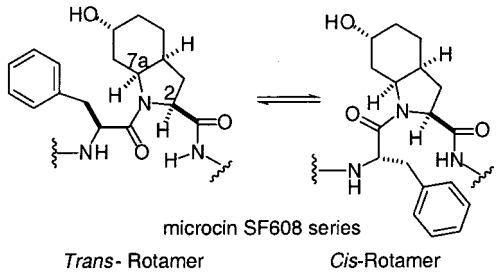


FIGURE 3. Amide isomer equilibrium of L-Choi derivatives (cf. Table 1).

Both synthetic and natural microcin SF608 show two peaks in their analytical HPLC profiles, in an approximate ratio of 3:1, and show two set of signals in their NMR spectra. These data are consistent with the existence of slow cis−trans rotational isomerism around the peptide bond preceding Choi (L-Phe-L-Choi). Such rotational isomerism is not commonly observed in chromatography, although the phenomenon is not without precedent, particularly for tertiary amide-containing peptides.[17] Rotational isomerism is, however, common enough on the time scale of NMR experiments and is often observed in spectroscopic studies on peptides and proteins.

NMR Studies about the Amide Isomer Equilibrium of the L-Phe-L-Choi Bond. Several interesting observations emerge from the ^{1}H NMR data recorded from L-Choi derivatives **8**−**10** and microcin SF608 (**1**). High-field NMR spectroscopy of these compounds indicates the presence of two predominant conformations both in $CDCl_3$ and DMSO-d_6 in a variable ratio (see Experimental Section). The assigned isomer geometry was based on NOESY experiments, and the chemical shift values for the signals of H-2 and H-7a of L-Choi. In the ^{1}H NMR spectra of **1** and its precursors (**8**−**10**) the H-2 and H-7a protons appear more deshielded in the trans isomers than in the cis isomers (e.g., δ 4.23 and 4.41 for trans rotamer and δ 3.90 and 4.09 for cis rotamer in **1**) (Table 1).

The configuration of Xaa-L-Choi has a significant influence on the chemical shifts of H-2 and H-7a of Choi. When the configuration is L, as in microcin SF608, a steric compression of the side chain of Xaa upon the Choi ring (specially at H-7a) is present in the trans rotamer (Figure 3) while when the configuration is D, as in the aeruginosins, the compression (specially at H-2) appears in the cis rotamers. Consequently, in the microcin SF608 series[18] H-7a is downfield in the trans rotamers while in the aeruginosin series H-2 is downfield in the cis rotamers, which agrees with the D configuration of the amino acid attached to the L-Choi.[19] Thus, taking into account all data reported so far about L-Choi containing peptides, the NMR signals of H-2 and H-7a of L-Choi can be used to determine the configuration of the core dipeptide of natural compounds that embody the L-Choi unit as well as to assign the cis−trans rotamers.[20]

In summary, the first total synthesis of microcin SF608 has been reported, and its NMR data have been compared with those of aeruginosins to give the NMR patterns for aquatic peptides containing the L-Choi motif.

(17) Jou, G.; González, I.; Albericio, F.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **1997**, *62*, 354−366.

(18) The anistrotopic effects of the aromatic ring of the phenylalanine residue undoubtedly also contribute to the low chemical shift for H-2 Choi in the cis rotamers in microcin and its precursors. This phenomenon has been precedented in the proline series: Poznanski, J.; Ejchart, A.; Wierzchowski, K. L.; Ciurak, M. *Biopolymers* **1993**, *33*, 781−795.

(19) For steric effects on the amide isomer equilibrium of prolyl peptides, see: (a) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902−12908. (b) Keller, M.; Sager, C.; Dumy, P.; Schutkowski, M.; Fischer, G. S.; Mutter, M. *J. Am. Chem. Soc.* **1998**, *120*, 2714−2720. (c) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312−3321. (d) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

(20) For the influence of chirality of the preceding acyl moiety on the cis/trans ratio of the proline peptide bond, see: Breznik, M.; Grdadolnik, S. G.; Giester, G.; Leban, I.; Kikelj, D. *J. Org. Chem.* **2001**, *66*, 7044−7050. See also ref 19d.

Experimental Section

General. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions. Unless otherwise noted, analytical thin-layer chromatography was performed on SiO_2 (silica gel 60 F_{254}), and the spots were located with iodoplatinate reagent or 1% aqueous $KMnO_4$. Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230−240 mesh ASTM). Drying of organic extracts during workup of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvent was accomplished with a rotatory evaporator. Chemical shifts of ^1H and ^{13}C NMR spectra are reported in ppm downfield (δ) from Me_4Si. The ^{13}C NMR spectra, when unambiguous assignation was not available, are reported as follows: chemical shift (multiplicity determined from DEPT spectra). Only noteworthy IR absorptions (cm^{-1}) are listed. Melting points were determined in a capillary tube. Microanalyses and HRMS were performed by the Centro de Investigación y Desarrollo (CSIC), Barcelona. Reversed-phase HPLC was performed using a Waters Nova-Pak C_{18} (15 × 0.39 cm, 4 μm, 60 Å) column and detection was by ultraviolet absorption at 238 nm. Abbreviations: NMM, (*N*-methylmorpholine); PyBOP (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

(*S*)-2-Acetyloxy-3-(4-benzyloxyphenyl)propionic Acid [AcO-L-Hpla(Bn)-OH] (2). To a solution of *O*-benzyl-L-tyrosine (2 g, 7.4 mmol) and NaOAc (2.2 g, 26.6 mmol) in glacial AcOH was slowly added isoamyl nitrite (3.7 mL, 27.4 mmol) at room temperature. The reaction mixture was stirred for 66 h. Hexane was added, and the mixture was concentrated to remove AcOH. The residue was dissolved in EtOAc (100 mL), and H_2O (70 mL) and concentrated HCl (20 mL) were added. The organic layer was washed with H_2O (5 × 30 mL) and extracted with saturated solution of $NaHCO_3$. The aqueous phase was acidified with HCl until pH 1 and extracted with EtOAc (3 × 30 mL). The dried organic extracts were concentrated, and the residue was purified by chromatography (1% AcOH in CH_2Cl_2) to give 1.5 g (64%) of acid **2**: R_f 0.50 (hexane/CH_2Cl_2/AcOH 3:6:1); mp 99−101 °C (H_2O); $[\alpha]_D$: −6.6 (*c* 0.6, $CHCl_3$); IR (KBr) 2926−3213, 1760, 1706, 1248, 1218; ^1H NMR (200 MHz, $CDCl_3$): 2.10 (s, 3H), 3.06 (dd, J = 14.6, 8.4 Hz, 1H), 3.20 (dd, J = 14.4, 4 Hz, 1H), 5.04 (s, 2H), 5.20 (dd, J = 8.6, 4.2 Hz, 1H), 6.92 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 7.34 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): 20.5 (q), 36.2 (t), 70.0 (t), 79.0 (d), 114.8 (d), 127.5 (d), 128.0 (d), 128.0 (s), 128.5 (d), 130.3 (d), 137.0 (s), 158.0 (s), 170.6 (s), 174.8 (s). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.77; H, 5.71. Found: C, 68.38; H, 5.81.

The optical rotation value corresponds to that of a crystallized sample, which was obtained as follows: (*S*)-(−)-1-phenylethylamine (0.09 mL, 0.70 mmol) was added to a solution of **2** (201 mg, 0.64 mmol) in $CHCl_3$ (3 mL), the mixture was stirred for 20 min, concentrated, and crystallized (Et_2O/EtOH) to give the corresponding salt of **2** (de ≥ 98%): ^1H NMR (300 MHz, $CDCl_3$) 1.56 (d, J = 6.6 Hz), 1.96 (s, 3H), 2.78 (dd, J = 14.5, 9.8 Hz, 1H), 2.93 (dd, J = 14.3, 3.1 Hz, 1H), 4.25 (q, J = 6.8 Hz, 1H), 4.89 (dd, J = 9.8, 3.6 Hz, 1H), 5.04 (s, 2H), 6.89 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 7.30−7.44 (m, 10H). A solution of the above crystals in CH_2Cl_2 (8 mL) was washed with 1 N HCl. The organic layer was dried and concentrated to give enantiopure **2**.

(*S*)-3-(4-Benzyloxyphenyl)-2-hydroxypropionic Acid (4). LiOH (0.1 N, 6.9 mL) was added to a cooled (0 °C) solution of **2** (72 mg, 0.23 mmol) in THF (2 mL), and the mixture was stirred at room temperature for 20 h. The mixture was extracted with Et_2O (2 × 4 mL), and the aqueous phase was acidified with 1 N HCl to pH 1. Extraction of the aqueous layer with $CHCl_3$/*i*PrOH (4:1, 4 × 10 mL) gave an organic extract which provided **4** as a white solid (62 mg, 99%); R_f 0.42 (EtOAc/MeOH 1:1); $[\alpha]_D$: −12.2 (*c* 0.62, MeOH); mp 140−142 °C; IR (KBr): 3448−2928, 1727, 1253; ^1H NMR (200 MHz, $CDCl_3$+CD_3OD): 2.87 (dd, J = 13.8, 7 Hz, 1H), 3.08 (dd, J = 14.2, 4 Hz, 1H), 4.36 (dd, J = 7.0, 4.4 Hz, 1H), 5.04 (s, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 7.41 (m, 5H), ^{13}C NMR (50 MHz, $CDCl_3$+CD_3OD): 40.7 (t), 70.8 (t), 72.8 (d), 115.6 (d), 128.5 (d), 128.7 (d), 129.4 (d), 131.0 (s), 131.5 (d), 138.8 (s), 158.9 (s), 177.2 (s). Anal. Calcd for $C_{16}H_{16}O_4 \cdot {}^1/_2H_2O$: C 68.30, H 6.05. Found: C 68.22, H 6.24.

(*S*)-2-Hydroxy-3-(4-hydroxyphenyl)propionic Acid [(−)-β-(4-Hydroxyphenyl)lactic Acid, l-Hpla] (5). Pd/C (10%, 12 mg) was added to a solution of **4** (63 mg, 0.23 mmol) in EtOAc/MeOH 1% (1 mL), and the mixture was stirred under atmospheric hydrogen pressure for 20 h. The catalyst was removed by filtration through a short pad of Celite, which was washed with MeOH (15 mL). The filtrate and washings were concentrated to give L-Hpla **5** (30 mg, 70%) as a white solid, which was recrystallized with $CHCl_3$/MeOH (9:1) to give white needles: R_f 0.42 (EtOAc/MeOH 1:1); mp 158−160 °C; $[\alpha]_D$: −10 (*c* 0.58, MeOH) IR (KBr): 3251, 1737, 1236; ^1H NMR: (200 MHz, $CDCl_3$+CD_3OD): 2.90 (dd, J = 14, 8 Hz, 1H, H-3), 3.10 (dd, J = 14.4, 4.4 Hz, 1H, H-3), 3.4 (br s, 1H, OH), 4.34 (dd, J = 7.8, 4.4 Hz, 1H, H-2), 6.75 (d, J = 8.4 Hz, 2H, H-6 and H-8), 7.10 (d, J = 8.8 Hz, 2H, H-5 and H-9); ^{13}C NMR (50 MHz, $CDCl_3$+CD_3OD): 40.7 (C-3), 73.0 (C-2), 116.0 (C-6 and C-8), 129.5 (C-4), 131.5 (C-5 and C-9), 157.0 (C-7), 177.5 (C-1). Anal. Calcd for $C_9H_{10}O_4 \cdot 1/2H_2O$: C 56.53, H 5.76. Found: C 56.56, H 6.02.

Boc-L-Phe-L-Choi-OMe (8a) and Its Trifluoroacetate 8b. Trifluoroacetic acid (5 mL) was added to a solution of **7**[4b] (391 mg, 1.31 mmol) in CH_2Cl_2 (10.6 mL). The reaction mixture was stirred at 0 °C for 2 h and concentrated. To a solution of the resulting material and Boc-L-Phe-OH (452 mg, 1.7 mmol) in CH_2Cl_2 (26 mL), cooled at 0 °C, PyBOP (885 mg, 1.7 mmol) and NMM (462.2 mg, 4.57 mmol, 0.55 mL) were added. After the solution had been stirred for 30 min at 0 °C and 22 h at room temperature, CH_2Cl_2 (30 mL) was added, and the solution was washed with 5% aqueous $NaHSO_4$ (3 × 25 mL), saturated aqueous $NaHCO_3$ (3 × 25 mL), and brine (1 × 15 mL). The organic layer was dried and concentrated, and the residue was purified by chromatography (hexane/EtOAc 1:1). The first eluate gave dipeptide **8b** (358 mg, 0.66 mmol) as a colorless syrup, and then a mixture of **8b** and **8a** (1:1 ratio, 20 mg) was eluted. A later elution gave dipeptide **8a** (225 mg, 0.51 mmol) as a white foam, the combined yield being 92%.

Compound 8a: R_f = 0.47 (EtOAc); $[\alpha]_D$: −37.7 (*c* 0.35, $CHCl_3$); IR (film): 3432−3304, 1748, 1710, 1634; ^1H NMR (500 MHz, $CDCl_3$, COSY, NOESY): trans-cis rotamer 1.6:1 ratio, trans rotamer: 1.30 (s, 9H, $C(CH_3)_3$), 1.47 (m, 4H, H-7, 2H-5 and H-4 Choi), 1.77 (m, 1H, H-7 Choi), 1.88 (ddd, J = 13, 12.5, 10 Hz, 1H, H-3 Choi), 2.11 (m, 2H, H-3 and H-4 Choi), 2.38 (dddd, J = 13, 6.5, 6.5, 6.5 Hz, 1H, H-3a Choi), 2.85 (dd, J = 14, 8 Hz, 1H, H-3 Phe), 3.10 (dd, J = 14, 6 Hz, 1H, H-3 Phe), 3.24 (br, 1H, OH-6 Choi), 3.71 (s, 3H, CO_2Me), 3.98 (br s, 1H, H-6 Choi), 4.42 (dd, J = 10.5, 8.5 Hz, 1H, H-2 Choi), 4.42 (m, 1H, H-7a Choi), 4.60 (ddd, J = 8, 8, 6 Hz, 1H, H-2 Phe), 5.33 (d, J = 9 Hz, 1H, NH); cis rotamer: 1.40 (s, 9H, $C(CH_3)_3$), 2.27 (m, 1H, H-3a Choi), 2.84 (dd, J = 13.5, 8 Hz, 1H, H-3 Phe), 2.97 (dd, J = 13.5, 5.5 Hz, 1H, H-3 Phe), 3.34 (dd, J = 8.5, 8.5 Hz, 1H, H-2 Choi), 3.69 (s, 3H, CO_2Me), 4.02 (br s, 1H, H-6 Choi), 4.23 (ddd, J = 8, 8, 6 Hz, 1H, H-2 Phe), 4.30 (ddd, J = 12, 6, 6 Hz, 1H, H-7a Choi), 5.46 (d, J = 9 Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, HMQC): trans−cis rotamer 2.5:1 ratio, trans rotamer: 18.9 (C-4 Choi), 25.6 (C-5 Choi), 28.2 ($C(CH_3)_3$), 30.3 (C-3 Choi), 34.0 (C-7 Choi), 36.9 (C-3a Choi), 39.3 (C-3 Phe), 52.1 (CO_2Me), 52.6 (C-2 Phe), 54.3 (C-7a Choi), 59.0 (C-2 Choi), 65.3 (C-6 Choi), 79.6 ($C(CH_3)_3$), 126.5 (C-7 Phe), 128.1 (C-6,6' Phe), 129.6 (C-5,5' Phe), 136.5 (C-4 Phe), 170.5 (C-1 Phe), 172.6 (CO_2Me); cis rotamer: 18.9 (C-4 Choi), 25.8 (C-5 Choi), 28.1 ($C(CH_3)_3$), 29.5 (C-3 Choi), 34.0 (C-7 Choi), 34.7 (C-3a Choi), 41.2 (C-3 Phe), 52.7 (C-2 Phe), 54.0 (C-7a Choi), 58.5 (C-2 Choi), 65.3 (C-6 Choi), 79.2 ($C(CH_3)_3$), 126.7 (C-7 Phe), 128.3 (C-6,6' Phe), 129.2 (C-5,5' Phe), 136.2 (C-4 Phe),

154.4 (NCO_2), 170.2 (C-1 Phe), 172.6 (CO_2Me). Anal. Calcd for $C_{24}H_{34}N_2O_6$: C 64.55, H 7.67, N 6.27. Found: C 64.44, H 7.93, N 6.03.

Compound 8b: R_f = 0.59 (EtOAc); $[\alpha]_D$: −34.3 (*c* 0.4, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) (1.3:1 mixture of trans and cis rotamers): trans rotamer: 1.37 (s, 9H), 1.40−1.99 (m, 6H), 2.48 (dddd, *J* = 13, 6.5, 6.5, 6.5 Hz, 1H), 2.86 (dd, *J* = 13.6, 6.6 Hz, 1H), 3.09 (dd, *J* = 13.6, 7.8 Hz, 1H), 3.74 (s, 3H), 4.36 (m, 3H), 5.14 (br s, 1H, H-6 Choi), 5.24 (d, *J* = 8.8 Hz, 1H), 7.12−7.23 (m, 5H); cis rotamer: 1.43 (s, 9H), 2.21 (dddd, *J* = 13, 6.5, 6.5, 6.5 Hz, 1H), 5.24 (br s, 1H), 5.40 (d, *J* = 8.8 Hz, 1H);^{13}C NMR (200 MHz, $CDCl_3$): (1.3:1 mixture of trans and cis rotamers): trans rotamer: 19.1 (t), 22.6 (t), 28.1 (q), 29.9 (t), 36.3 (d), 39.1 (t), 52.0 (q), 53.2 (d), 53.6 (d), 58.9 (d), 74−3 (d), 79.2 (s), 126.5−129.2 (doublets), 136.2 (s), 154.2 (s), 170.3−172.4 (singlets); cis rotamer: 19.0 (t), 22.9 (t), 27.9 (q), 34.1 (d), 41.2 (t), 58.3 (d), 74.2 (d), 79.4 (s), 136.1 (s), 154.7 (s).

AcO-L-Hpla(Bn)-L-Leu-L-Choi-OMe (9a) and Its Trifluoroacetate 9b. TFA (2 mL, 53 mmol) was added to a cooled (0 °C) solution of **8b** (350 mg, 0.65 mmol) in CH_2Cl_2 (9 mL), and the mixture was stirred for 50 min and concentrated. A solution of the resulting material in CH_2Cl_2 (3.6 mL) with NMM (108.34 μL, 0.97 mmol) was added to a cooled (0 °C) solution of the L-Hpla derivative **2** (245 mg, 0.78 mmol) in CH_2Cl_2 (4.3 mL), which had been previously stirred at this temperature with PyBOP (406 mg, 0.78 mmol) and NMM ((153 μL, 1.3 mmol) for 30 min. The mixture was stirred at room temperature for 22 h. CH_2Cl_2 (20 mL) was added, and the organic layer was successively washed with HCl (1 N, 3 × 25 mL), saturated $NaHCO_3$ solution (3 × 25 mL), and brine (1 × 10 mL). The concentrated dried extract was purified by chromatography (hexane/EtOAc 1:1) to give the trifluoroacetate **9b** (236 mg) and then the alcohol **9a** (120 mg), both as white solids, the combined yield being 78%.

Compound 9a: mp 81−84 °C; $[\alpha]_D$: −39.4 (*c* 0.5, $CHCl_3$); IR (film): 3417−3296, 1746, 1627; ^{1}H NMR (500 MHz, DMSO, COSY, NOESY) trans−cis rotamer 10:1 ratio, trans rotamer: 1.39 (m, 5H, 2H-5, 2H-4, H-7 Choi), 1.51 (br d, *J* = 13.5 Hz, 1H, H-7 Choi), 1.82 (ddd, *J* = 12.5, 12, 11 Hz, 1H, H-3β Choi), 1.93 (s, 3H, CH_3CO), 2.09 (ddd, *J* = 12.5, 7.5 7 Hz, 1H, H-3α Choi), 2.18 (dddd, *J* = 12, 6, 6, 6 Hz, 1H, H-3a Choi), 2.76 (dd, *J* = 14.5, 9 Hz, 1H, H-3 Phe), 2.84 (dd, *J* = 14.5, 8 Hz, 1H, H-3 Hpla), 2.89 (dd, *J* = 14.5, 4.5 Hz, 1H, H-3′ Hpla), 2.94 (dd, *J* = 14, 6 Hz, 1H, H-3 Phe), 3.62 (s, 1H, OMe), 3.80 (br s, 1H, H-6 Choi), 4.26 (dd, *J* = 10, 8.5 Hz, 1H, H-2 Choi), 4.42 (ddd, *J* = 12.5, 6, 6 Hz, 1H, H-7a Choi), 4.50 (d, *J* = 2.5 Hz, 1H, OH-6 Choi), 4.57 (ddd, *J* = 8, 8, 6 Hz, 1H, H-2 Phe), 5.03 (dd, *J* = 8.5, 4 Hz, 1H, H-2 Hpla), 5.04 (s, 2H, CH_2Ar), 6.88 (d, *J* = 8.5 Hz, 2H, H-6 and H-8 Hpla), 7.07 (d, *J* = 8.5 Hz, 2H, H-5 and H-9 Hpla), 7.29 (m, 10H, H-5, 5′, 6, 6′, 7 Phe and Ar), 8.53 (d, *J* = 7.5 Hz, 1H, NH); cis rotamer: 1.94 (s, 3H, CH_3CO), 3.58 (s, 3H, OMe), 3.68 (dd, *J* = 8.5, 8.5 Hz, 1H, H-2 Choi), 3.83 (br s, 1H, H-6 Choi), 4.14 (ddd, *J* = 12.5, 6, 6 Hz, 1H, H-7a Choi), 4.31 (m, 1H, H-2 Phe), 5.11 (dd, *J* = 8.5, 3.5 Hz, 1H, H-2 Hpla), 6.88 (d, *J* = 8 Hz, 2H, H-6, H-8 Hpla), 7.07 (d, *J* = 8 Hz, 2H, H-5, H-9 Hpla), 8.29 (d, *J* = 9 Hz, 1H, NH);^{13}C NMR (75 MHz, $CDCl_3$, HMQC): trans−cis rotamer 4:1 ratio, trans rotamer: 18.9 (C-4 Choi), 20.9 (CH_3CO), 25.8 (C-5 Choi), 30.3 (C-3 Choi), 34.0 (C-7 Choi), 36.8 (C-3 Phe), 36.9 (C-3a Choi), 39.0 (C-3 Hpla), 50.8 (C-2 Phe), 52.1 (CO_2-Me), 54.6 (C-7a Choi), 59.1 (C-2 Choi), 65.5 (C-6 Choi), 69.8 (OCH_2Ar), 74.0 (C-2 Hpla), 114.5 (C-6 and C-8 Hpla), 127.4−129.8 (C-5, 5′, 6, 6′, 7 Phe and Ar), 128.0 (C-4 Hpla), 130.5 (C-5 and C-9 Hpla), 135.7 (C-7 Phe, *ipso*-Ar), 136.8 (C-4 Phe), 157.6 (C-7 Hpla), 168.4, 169.2, 169.5, 172.4 (CO); cis rotamer: 18.9 (C-4 Choi), 25.8 (C-5 Choi), 29.6 (C-3 Choi), 31.9 (C-7 Choi), 34.7 (C-3a Choi), 36.8 (C-3 Phe), 40.5 (C-3 Hpla), 51.5 (C-2 Phe), 53.0 (CO_2Me), 54.1 (C-7a Choi), 58.3 (C-2 Choi), 65.5 (C-6 Choi), 74.0 (C-2 Hpla), 114.5 (C-6,8 Hpla), 127.4−129.8 (C-5.5′,6,6′, 7-Phe, Ar), 130.5 (C-5, 9 Hpla), 135.8 (*ipso*-Ar), 136.8 (C-4 Phe), 157.6 (C-7 Hpla), 167.8, 169.3, 169.4 (CO). Anal. Calcd for $C_{37}H_{42}N_2O_8 \cdot H_2O$: C 67.25, H 6.41, N 4.23. Found: C 67.68, H 6.89, N 4.22.

Compound 9b: mp 48−51 °C; $[\alpha]_D$: −34 (*c* 0.45, $CHCl_3$); ^{1}H NMR (200 Mz, $CDCl_3$) (1.7:1 mixture of trans and cis rotamers): trans rotamer: 1.39−2.25 (m, 8H), 2.04 (s, 3H), 2.44 (dddd, *J* = 12, 6, 6, 6 Hz, 1H), 2.64−3.12 (m, 4H), 3.74 (s, 3H), 4.34 (m, 1H), 4.42 (dd, *J* = 10, 8 Hz, 1H), 4.71 (ddd, *J* = 8, 8, 6 Hz, 1H), 5.01 (s, 2H), 5.11 (br s, 1H); 5.31 (dd, *J* = 6, 4 Hz, 1H), 6,57 (d, *J* = 8.4 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 8,8 Hz, 2H), 7.32 (m, 10H); cis rotamer: 2.11 (s, 3H), 2.64−3.12 (m, 4H), 3.73 (s, 3H), 5.23 (br s, 1H), 6.75 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) (2:1 mixture of trans and cis rotamers): trans rotamer: 19.2 (t), 20.8 (q), 22.7 (t), 30.0 (t), 30.4 (t), 36.3 (d), 36.7 (t), 39.9 (t), 51.4 (d), 52.2 (q), 53.9 (t), 58.1 (d), 69.7 (t), 73.9 (d), 114.4 (d), 126.9−129.3 (doublets), 130,5 (d), 135.6 (s), 135.7 (s), 136.7 (s), 157.6 (s), 168.3 (s), 169.1 (s), 169.5 (s), 169.6 (s), 172.3 (s); cis rotamer: 20.9 (q), 22.9 (t), 34.2 (d), 40.5 (t), 51.5 (d), 53.0 (q), 53.4 (d), 58.2 (d), 74.2 (d), 114.5 (d), 126.9−129.3 (doublets), 156.1 (s).

HO-L-Hpla(Bn)-L-Phe-L-Choi-diBoc-Agma (10). A cooled solution of LiOH (0.1 N, 4.2 mL, 0.42 mmol) was added dropwise to a cooled (0 °C) solution of peptide **9a** (89 mg, 0.14 mmol) in THF (3.5 mL). After having been stirred at room temperature for 20 h, the solution was washed with Et_2O (2 × 7 mL) and acidified to pH 1 with 1 N HCl. The aqueous solution was extracted with $CHCl_3$/*i*PrOH (4:1, 6 × 7 mL). The combined extracts were washed with brine, dried, and concentrated to give the corresponding acid (70 mg, 86%) as a white solid (structure not shown); R_f 0.47 (EtOAc/MeOH 1:1); ^{1}H NMR (200 MHz, $CDCl_3$+drops CD_3OD): trans-cis rotamer 10:1 ratio, trans rotamer: 1.27−2.45 (m, 9H), 2.53−3.14 (m, 4H), 3.38−4.88 (m, 5H), 5.02 (s, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.21−7.39 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$+drops CD_3OD): trans−cis rotamer 10:1 ratio, trans rotamer: 18.8 (t), 25.5 (t), 30.2 (t), 33.7 (t), 36.7 (d), 38.6 (t), 39.4 (t), 51.0 (d), 54.9 (d), 59.2 (d), 65.1 (d), 69.9 (t), 72,5 (d), 114.5 (d), 126.8−129.5 (doublets), 130.3 (d), 135.5 (s), 135.7 (s); 136.8 (s); 157.3 (s), 170.5 (s), 173.5 (s), 174.0 (s); cis rotamer: 29,6 (t), 34.7 (t), 51.4 (d), 54.2 (d), 58.5 (d), 130.5 (d).

PyBOP (62.5 mg, 0.12 mmol) and NMM (34 μL, 0.30 mmol) were added to a cooled (0 °C) solution of the above acid (70 mg, 0.12 mmol) and agmatine **6**[15] (56 mg, 0.16 mmol) in DMF (2 mL). After the solution had been stirred for 30 min at 0 °C and 22 h at room temperature, EtOAc (70 mL) was added, and the solution was washed with saturated aqueous $NaHCO_3$ (1 × 50 mL) and H_2O (5 × 60 mL). The organic layer was dried and concentrated, and the residue was purified by chromatography (EtOAc/MeOH 95:5) to give **10** (83 mg, 77%) as a white foam: R_f 0.77 (EtOAc/MeOH 1:1); $[\alpha]_D$: −24.2 (*c* 0.65, $CHCl_3$); IR (film): 3333, 1636; ^{1}H NMR (200 MHz, $CDCl_3$) 1.46−2.4 (m, 13H), 1.47 and 1.49 (2s, 9H each), 2.6−3.4 (m, 8H), 4.02 (br s, 1H), 4.24 (br, 1H), 4.4 (m, 2H), 4.89 (ddd, *J* = 8, 8, 6 Hz, 1H), 5.02 (s, 2H), 6.9 (d, *J* = 8.6 Hz, 2H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.1−7.4 (m, 12H), 8.3 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 19.0 (t), 25.8 (t), 26,5 (t), 26.8 (t), 28.0 (q), 28.3 (q), 29.7 (t), 33.4 (t), 36.5 (d), 39.1 (t), 39.4 (t), 39.7 (t), 40.5 (t), 51.5 (d), 55.4 (d), 59.9 (d), 65.8 (d), 69.9 (t), 72.8 (d), 79.2 (s), 83.0 (s), 127−130.5 (doublets), 135.8 (s), 136.8 (s), 153.1 (s), 156.0 (s), 157.6 (s), 163.4 (s), 170.7 (s), 172.8 (s). Anal. Calcd for $C_{49}H_{66}N_6O_{10}$: C 65.47, H 7.35, N 9.35. Found: C 65.18, H 7.56, N 9.11.

L-Hpla-L-Phe-L-Choi-Agm [Microcin SF608] (1). A solution of **10** (32 mg, 0.035 mmol) in CH_3CN (3 mL) and 6 N HCl (0.6 mL) was stirred at room temperature for 6 h, then concentrated to give the hydrochloride of the intermediate L-Hpla(Bn)-L-Phe-L-Choi-Agm, as an amorphous solid. To a solution of this tetrapeptide (27 mg) in EtOAc/MeOH (1:1, 3 mL) was added Pd/C (10%, 16.5 mg and an additional 27 mg after 6 h), and the mixture was stirred under hydrogen atmospheric pressure for 24 h. The catalyst was removed by filtration with Celite, which was washed with MeOH (30 mL).

The organic solution was concentrated to give microcin SF608 (**1**) (25 mg, 99%); RP-HPLC t_R 1.13 min (cis rotamer) and 1.47 min (trans rotamer), isocratic elution with A/B 60:40 for 30 min, where A is H_2O and B is CH_3CN/0.05% TFA; coelution of **1** with natural microcin SF608 gave the same two peaks; $[\alpha]_D$: −27.4 (*c* 1.25, MeOH). The ^1H NMR (500 MHz, DMSO-d_6, COSY, NOESY), trans-cis rotamer 3:1 ratio, and ^{13}C NMR (100 MHz, DMSO-d_6, HSQC) data of **1** matched those reported in the literature for the natural product.[2a,16] FABMS for $C_{32}H_{44}N_6O_6$: 609.3 ($[M + H]^+$, 100), 567.2 (12), 461.2 (10), 383.3 (40).

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