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Natural dolapyrrolidone: Isolation and absolute stereochemistry of a substructure of bioactive peptides

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Abstract

During the course of our chemical analysis of the hydrophilic fractions from marine cyanobacterium *Moorena producens*, we have isolated natural dolapyrrolidone (Dpy, **1**), a natural pyrrolidone derived from phenylalanine, for the first time as a single compound. Compound **1**, with an (*S*)-L absolute stereochemistry, was previously identified as a substructure that is common among several bioactive natural peptides. Surprisingly, the absolute stereochemistry of the isolated natural **1**, determined through total synthesis, was (*R*)-D. This result was unambiguously determined by HPLC analysis using a chiral stationary column by comparing the retention times of the natural **1** and authentic samples of synthetic enantiomers. To verify the unexpected result, the absolute stereochemistry of the natural **1** was confirmed by X-ray crystallographic analysis of Pt-complex derivative using the synthetic enantiomer.

KEYWORDS

amino acid, circular dichroism, HPLC analysis, Pt complex, total synthesis, X-ray crystallographic analysis

1 | INTRODUCTION

Marine cyanobacteria[1] are the ubiquitous elements of marine ecosystem worldwide and have attracted a great deal of interest as sources of important natural products. Aplysiatoxins were isolated from the marine cyanobacterium *Moorena producens* (formerly known as *Moorea producens*[2] and *Lyngbya majuscula*) as the causative agents of a form of contact dermatitis (swimmers' itch).[3] This finding was soon followed by isolation of another irritant lyngbyatoxin A.[4] One of the authors also revealed that food contaminated with *M. producens* caused food poisoning by producing aplysiatoxins.[5] Research on *M. producens* has recently provided some new indole derivatives.[6] During the course of this research, an interesting amino acid derivative, dolapyrrolidone (Dpy, **1**), was isolated. This phenylalanine derivative **1** has long been known as the common substructure of several bioactive peptides, such as dolastatin 15,[7] but had not been isolated as a single compound. The absolute stereochemistry of natural Dpy **1** was indirectly determined to be (*S*)-L by the synthesis of dolastatin 15 that showed the same potent ED_{50} activity with the natural product, whose seven amino acids were all supposed to have L-

configuration. Herein, we report the experimental details for isolation, structure elucidation, synthesis, and determination of the absolute stereochemistry of this long-known compound **1** by comparison with synthetic authentic samples on HPLC analysis using chiral stationary phase (chiral HPLC). The stereochemistry of synthetic **1** is unambiguously determined by X-ray crystallographic analysis (X-ray analysis) using Pt-complexation method.

2 | MATERIALS AND METHODS

2.1 | General

NMR spectral data were obtained by a Bruker AVANCE III 800-MHz spectrometer (Bruker Co., Bremen, Germany) for natural Dpy structural determination and a JEOL JNM-alpha 400-MHz spectrometer (JEOL, Tokyo, Japan) for synthetic intermediates and products. Infrared spectra (IR) were recorded on a Fourier transfer infrared spectrophotometer (Perkin Elmer Spectrum One) by using a diffuse reflector for crystalline samples. UV spectrum was recorded on a Shimadzu UV-2500PC with a 2-mm quartz cell. Electronic circular dichroism (ECD) spectra were taken on a JASCO J-1500 spectrometer, in which nitrogen flow for measurements at wavelength of less than 200 nm was kept at 15- to 20-L/min level. Optical rotations were measured on a JASCO P-2200 polarimeter with a 5-cm cell. Mass spectra were recorded on Bruker microTOF QII mass spectrometer for natural Dpy and a Thermo Fischer Scientific LTQ Orbitrap XL of Hiroshima University Natural Science Center for Basic Research and Development (N-BARD) for synthetic intermediates and products, unless otherwise mentioned. Melting point was taken on a Yanako MP-S3 apparatus and was uncorrected. X-ray crystallographic data was collected using a Rigaku Saturn 724 CCD diffractometer with graphite monochromated Mo-Kα radiation ($\lambda = 0.7107$ Å) at 123 K. Commercially available reagents were used as received. Solvents were purified over appropriate drying agents before use.

2.2 | Plant material

The cyanobacterium *M. producens* was collected from the internal zone of Kuba, Nakagusuku Nakagami District, Okinawa Prefecture, Japan, in July 2010. The sample was immediately transferred to the Okinawa Prefectural Institute of Public Health and Environment and preserved in a freezer at −30°C. The frozen sample was sent to Tokyo University of Marine Science and Technology and kept in a freezer at −30°C until extraction. A voucher specimen (#10/7/13-1), identified as *M. producens* as reported,[5] has been retained by the author (H. N.).

2.3 | Extraction and isolation

The extract was prepared from 866 g of the cyanobacterium *M. producens* as previously reported.[6] The solvents used for the extractions were ethanol, methanol (MeOH), acetone, and water (5 L of each solvent, three times). The extract was evaporated to obtain the residues (37.8 g, dry wet.). The residue suspended in 80% MeOH with 20% water (3 L) was extracted three times with *n*-hexane (3 L) to get the *n*-hexane extract (2.37 g, dry wet), and the defatted residue (185.2 g, dry wet) was extracted three times with ethyl acetate (EtOAc, 3 L) to get the EtOAc extract (1.65 g, dry wet), which was washed with water, and the residual small particles were removed by filtration. The EtOAc extract was separated by a glass open column (20 × 300 mm) conducted on ODS (ODS-7515-12A, 5 μm, Senshu Scientific Co., Japan) by stepwise elusion. The eluting solvent was passed through the column by gravity in sequence obtaining five fractions: 50% MeOH (81.5 mg), 70% MeOH (299 mg), 90% (906 mg), 100% MeOH (133 mg), and 20% formic acid (80% MeOH) (35.2 mg). To obtain more separated fractions, the 70% MeOH fraction was further passed through an open glass column by the following gravity in sequence: 50% MeOH (360 mg), 52.5% MeOH (80 mg), 55% MeOH (80 mg), 57.5% MeOH (130 mg), 60% MeOH (110 mg), 62.5% MeOH (130 mg), 65% MeOH (160 mg), 67.5% MeOH (70 mg), 70% MeOH (60 mg), 72.5% MeOH (30 mg), 100% MeOH (90 mg), and 5% formic acid in MeOH (30 mg). The 50–70% MeOH fraction was purified using reverse-phase HPLC (COSMOSIL C18 K12671, 20 × 250 mm, 5 μm, Nacalai Tesque, ODS) with isolating condition of 50% MeOH isocratic from 0 to 5 min, 50% MeOH to 100% MeOH gradient from 5 to 20 min, and 100% MeOH from 20 to 40 min, flow rate 8.0 ml/min, observed at 210-nm UV absorbance to obtain 15 fractions. The most abundant 10th fraction (37.3 mg, dry wet) was further purified using an ODS-recycle HPLC (COSMOSIL PACKED COLUMN C18 K54116, 10 × 250 mm, Nacalai Tesque) (65% MeOH, observed at 210 nm, 1.0 ml/min) to isolate 1.61 mg of dolapyrrolidone **1**, $[\alpha]_D^{24}$–9.0 (c 0.11, MeOH).

2.4 | Preparation of Boc-dolapyrrolidone ((*S*)-L-4)

A solution of *t*-butyloxycarbonyl-L-phenylalanine (Boc-L-Phe-OH) (512 mg, 1.93 mmol), Meldrum's acid (345 mg,

2.39 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (455 mg, 2.37 mmol), and 4-dimethylaminopyridine (DMAP) (458 mg, 3.73 mmol) in CH_2Cl_2 (4.0 ml) was stirred at room temperature overnight. Following the filtration of the reaction mixture, the CH_2Cl_2 solution was washed with water and brine. After drying the obtained solution with Na_2SO_4, the solvent was removed under vacuum to give the crude product as yellow solids. The obtained crude product was refluxed in EtOH for 3 h, giving the cyclized product (*S*)-L-**3** after removal of the solvent. The ESI-MS indicated that the cyclized product (*S*)-L-**3** was produced (*m/z* 288.12396 $[M - H]^-$ [288.12413 calculated for $C_{16}H_{18}NO_4$]), but the given crude product was directly used for the following reaction, as the impurity could not be removed under several purification conditions including HPLC of either normal or reverse phase.

To a mixture of the crude product of (*S*)-L-**3** (471 mg containing some impurity) and K_2CO_3 in acetone (5.0 ml) was added dimethyl sulfate (860 μl, 9.07 mmol) at −78°C. After stirring the reaction mixture overnight at room temperature, the solution was concentrated to give a sticky brown solid. The residue was purified by column chromatography on silica gel eluted with hexane-ethyl acetate (1:1 v/v) to afford (*S*)-L-Boc-Dpy **4** (143 mg, 24.5% for two steps) as brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.63 (9H, s), 3.15 (1H, dd, J = 2.8, 5.2 Hz), 3.47 (1H, dd, J = 3.0, 5.2 Hz), 3.79 (3H, s), 4.70 (1H, dd, J = 2.8, 3.0 Hz), 4.83 (1H, s), 7.02 (2H, d, J = 6.1 Hz), 7.24 (3H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3, 35.4, 59.5, 60.7, 82.5, 95.1, 126.6, 128.4, 129.4, 134.0, 149.4, 168.6, 176.0 ppm. ESI-MS: *m/z* 326.13672 $[M + Na]^+$ (326.13628 calculated for $C_{17}H_{21}NO_4Na$).

2.5 | Preparation of dolapyrrolidone ((*S*)-L-1)

To a solution of Boc-Dpy ((*S*)-L-**4**) (97.5 mg, 321 μmol) in CH_2Cl_2 (2.2 ml) was added trifluoroacetic acid (TFA) (500 μl, excess) at 0°C for 6 h to room temperature overnight. The solution was concentrated and purified by column chromatography with hexane-ethyl acetate (1:1 v/v) to give Dpy ((*S*)-L-**1**) (81.9 mg, almost quantitative). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.75 (1H, dd, J = 7.9, 13.7 Hz), 3.15 (1H, dd, J = 4.0, 13.7 Hz), 3.84 (3H, s), 4.31 (1H, dd, J = 4.0, 7.9 Hz), 5.03 (1H, s), 7.12 (2H, d, J = 6.7 Hz), 7.17 (1H, m), 7.26 (2H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.6, 58.8, 59.9, 92.8, 127.3, 128.7, 129.1, 135.1, 173.4, 176.4 ppm. IR (reflection, cm^{-1}): 3223, 2943, 1668, 1616, 1496, 1233, 1042, 730, 697. UV (CH_3CN): 209 nm (ε 25,000). ESI-MS: *m/z* 226.08351 $[M + Na]^+$ (226.08385 calculated for $C_{12}H_{13}NO_2Na$). Note that the product contained a little amount of unknown impurity, which was removed when purified with the chiral HPLC, $[\alpha]_D^{18}$ + 76 (c 1.0, $CHCl_3$), −36 (c 1.0, MeOH), −80 (c 0.1, MeOH) for (*R*)-D-**1**.

2.6 | Preparation of dolapyrrolidone-Pt complex (6)

To a solution of synthetic (*R*)-D-**1** (10.0 mg, 49.2 μmol) in CH_2Cl_2 (5.0 ml) was added *trans*-$PtCl_2$(2,4,6-trimethylpyridine)(ethylene) (**5**) (30.7 mg, 73.9 μmol) at room temperature. The reaction mixture was stirred for 95 h; thus, the solution was concentrated. The yield of the generated Pt complex (36%) was determined by ^{1}H NMR analysis of the crude mixture with 1,3,5-trimethoxybenzene as an internal standard, which was purified by gel permeation chromatography with chloroform. The isolated (*R*)-D-**6** was slowly decomposed to Dpy **1** in an organic solvent. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (3H, s), 2.76 (1H, dd, J = 10.0, 14.0 Hz), 3.26 (1H, dd, J = 3.6, 14.0 Hz), 3.38 (6H, s), 3.87 (3H, s), 4.37 (1H, dd, J = 3.2, 10.0 Hz), 5.27 (1H, d, J = 1.2 Hz), 6.88 (2H, s), 7.28–7.40 (5H, m), 7.67 (1H, brs) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 27.9, 38.1, 59.2, 61.1, 93.4, 124.0, 127.6, 129.2, 129.4, 135.9, 150.4, 161.6, 179.1, 180.2 ppm. IR (reflection, cm^{-1}): 3431, 3108, 2927, 1728, 1627, 1237, 1070, 726. EI-MS (JEOL JMS-700): *m/z* 589.0862 $[M]^+$ (589.0863 calculated for $C_{20}H_{24}Cl_2N_2O_2Pt$). The fraction containing (*R*)-D-**6** after the chromatography naturally afforded single crystals, mp 175°C, which were subjected to the X-ray crystal structure analysis. Deposition number of the Cambridge Crystallographic Data Center for compound (*R*)-D-**6** is CCDC 1974660. Selected crystallographic data: monoclinic, $P2_1$ (No. 4), a = 8.353(2) Å, b = 9.123(2) Å, c = 13.768(4) Å, β = 96.992(6)°, V = 1041.5(5) Å^3, Z = 2, R_1 = 0.0323, wR_2 = 0.0689, Flack parameter = −0.021(7).

3 | RESULTS AND DISCUSSION

Compound **1** was isolated as light brown amorphous. The HR-ESI-MS analysis of compound **1** revealed protonated adduct ion peaks at *m/z* 204.10293 $[M + H]^+$ (calculated for $C_{12}H_{14}NO_2$ 204.10245) and 407.19985 (calculated for $C_{24}H_{27}N_2O_4$ for 407.1971). The molecular formula of **1** was determined to be $C_{12}H_{13}NO_2$, indicating seven degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra were assigned by analysis of information from ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC spectra (Table 1 and Supporting Information). The ^{1}H NMR spectrum indicated that the compound **1** possessed a benzene

TABLE 1 NMR assignment of compound **1** ($CDCl_3$, AVANCE800)

No.	^{13}C	^{1}H (*J*/Hz)	COSY	HMBC (H → C)
C-1	173.6			
C-2	93.8	5.12 (1H, s)		1,4
C-3	177.6			
C-4	58.8	4.34 (1H, dd, 9.77, 3.44)	5	1, (2), 3, 5, 6
C-5	38.7	3.32 (1H, dd, 13.68, 3.33), 2.73 (1H, dd, 13.58, 9.52)	4	3, 4, 6, 7
C-6	136.5			
C-7	129.1	7.31 (2H, d, 7.56)	8	5, 9
C-8	128.8	7.43 (2H, t, 7.44)	7, 9	6
C-9	127.1	7.37 (1H, m)	8	7
C-10	58.4	3.95 (1H, s)		3
N-H		5.60 (1H, s)		

ring, an olefin, a methyl group that is connected to oxygen, and an ethyl group that is connected to either nitrogen or oxygen. The ^{13}C NMR suggested that the compound has either an amide or an ester. The ^{1}H-^{13}C HSQC was used to assign direct connection between carbons and hydrogens. The ^{1}H-^{1}H COSY confirmed the existence of one CH-CH_2 unit and one benzene ring by showing two sets of correlation sequence, H-4–H-5 and H-7–H-8–H-9. In the CH-CH_2 unit, the coupling constants between H-4 and two H-5 protons showed mutual interaction that has no other couplings, suggesting that they are both connected to quaternary carbons. In the benzene ring, the ^{1}H-^{13}C HMBC was helpful to reveal that C-6 (δ 136.5), an aromatic carbon, has correlation with H-8. Except for aromatic protons, the HMBC correlations to aromatic carbons were observed only from H-4 and H-5. Correlations were observed from H-4 to C-1, not from H-5, and from H-5 to C-7, not from H-4 (Figure 1A), indicating that C-5 is connected to C-6. The singlet three-proton peak at δ 3.95, which is directly connected to C-10, had an HMBC correlation only with C-3, which is connected to C-4 as the HMBC correlation was observed from H-5 to C-3.

To build the complete structure, three more concerns (the degree of unsaturation, the HMBC correlations from both H-2 and H-4 to the carbonyl C-1, and the connection between C-3 and the methoxy group [C-10]) were converged, leading to the single possible structure shown as **1** in Figure 1, dolapyrrolidone (Dpy). This structure **1** was named after dolastatin 15[7] and has been repeatedly referred to as the common substructure among such second metabolites as mycapolyols A–F,[8] belamide A,[9] caldoramide,[10] and smenamide A and B.[11] The crude EtOAc extract in this study was screened by LC–MS, capturing none of the reported Dpy-correlated metabolites. Therefore, Dpy (**1**) was isolated as the sole natural product for the first time.

The natural Dpy **1** is optically active, showing distinct Cotton effects (weak plus at 240 and strong minus at 216 nm, 113.6 μg/ml, acetonitrile). The optical rotation of the isolated **1** indicated a minus value (−9.0, c = 0.11, MeOH) the same sign as the one reported for (*S*)-L-**1** (−62.3, c = 1.0, $CHCl_3$),[11] but the measurement was not

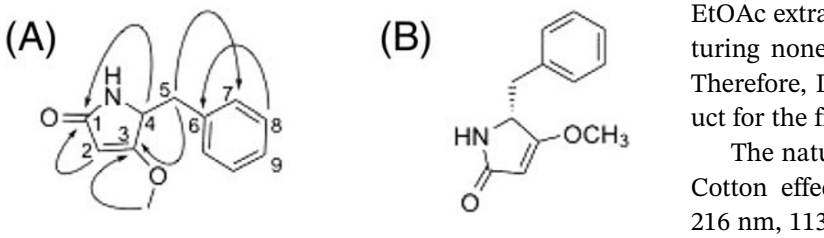
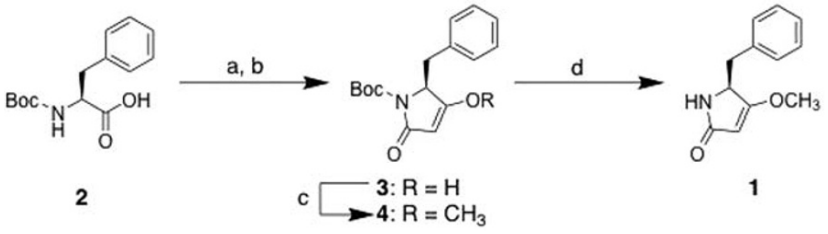


FIGURE 1 Structure of dolapyrrolidone (Dpy, **1**). (A) Plain structure of **1** and important HMBC correlations. (B) Structure of (*R*)-D-**1**



SCHEME 1 Total synthesis of dolapyrrolidone **1** (represented by (*S*)-L-form). Reagents and conditions: (a) EDC, DMAP, Meldrum's acid, CH_2Cl_2, RT, 22 h; (b) EtOH, reflux, 2 h; (c) $(CH_3)_2SO_4$, K_2CO_3, acetone, −78°C-RT, 14 h (24% from **2**); (d) TFA, CH_2Cl_2, 0°C-RT, 16 h (quant.)

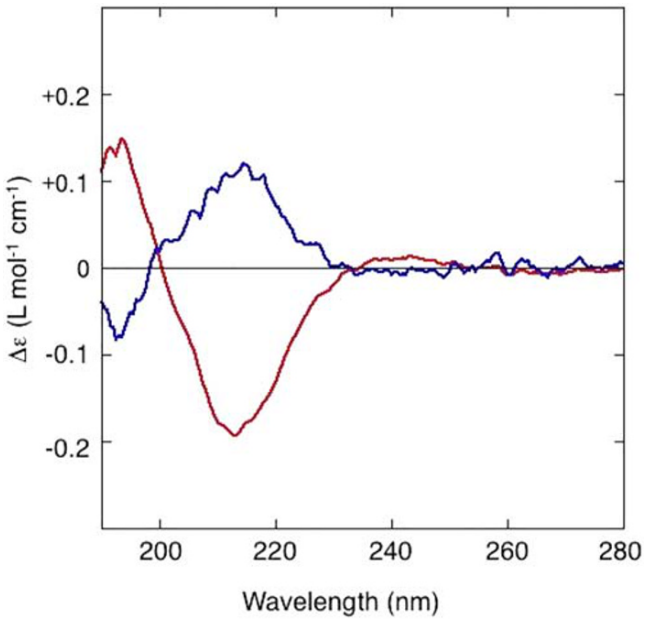


FIGURE 2 ECD spectra of synthetic dolapyrrolidones (Dpy, **1**) in acetonitrile. The curves of (*S*)-L- and (*R*)-D-**1** are shown by blue and red lines, respectively

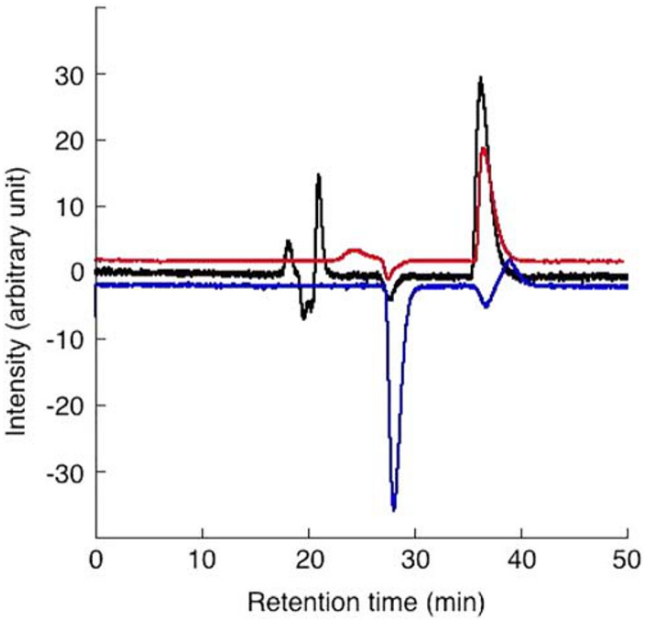


FIGURE 3 Chromatogram for natural (black line) and synthetic dolapyrrolidones (Dpys). (*R*)-D- (red) and (*S*)-L-**1** (blue) are superimposed for comparison. Elusion was observed by ECD detection (254 nm). Chiral HPLC analysis of natural Dpy (**1**) was carried out on CHIRALCEL OJ-H with eluent hexane/isopropyl alcohol (90:10 v/v)

deemed reliable for determining the absolute stereochemistry unambiguously, as it was measured from a small amount of natural product, which might include some unknown impurity. The absolute stereochemistry of the previously reported (*S*)-L-Dpy (**1**) was originally derived from the total synthesis of dolastatin 15,[7] a biologically active depsipeptide. We had accordingly anticipated the absolute stereochemistry of this isolated natural Dpy **1** to be (*S*)-L-form, corresponding to the absolute stereochemistry of L-phenylalanine, the natural amino acid. We first attempted a quantum mechanics calculation[12] for predicting the ECD spectra of both (*S*)-L- and (*R*)-D-Dpy-**1**. The result, however, did not provide satisfactory Cotton effects at 240 and 216 nm for a confident assignment. To determine the absolute stereochemistry of natural Dpy **1** convincingly, we carried out the total synthesis, enantiomer separation by a chiral HPLC, and X-ray analysis.

Syntheses of both (*S*)-L- and (*R*)-D-**1** were achieved as summarized in Scheme 1. A commercially available Boc-protected L-phenylalanine (Boc-L-Phe-OH) was converted to the corresponding pyrrolidone derivative **3** by using Meldrum's acid. After methylation of compound **3** at low temperature, Boc group was deprotected to give Dpy **1**. The enantio-excess of synthetic Dpy **1**, which was almost spoiled at room temperature, was remained in fact when methylation reaction was carried out at −78°C. According to the ECD spectra measured for both synthetic enantiomers as shown in Figure 2, the natural Dpy (ECD spectrum is shown in Figure 4) had the same absolute stereochemistry as (*R*)-D-**1**, indicating that the natural Dpy had the opposite absolute stereochemistry as compared to the natural L-amino acid.

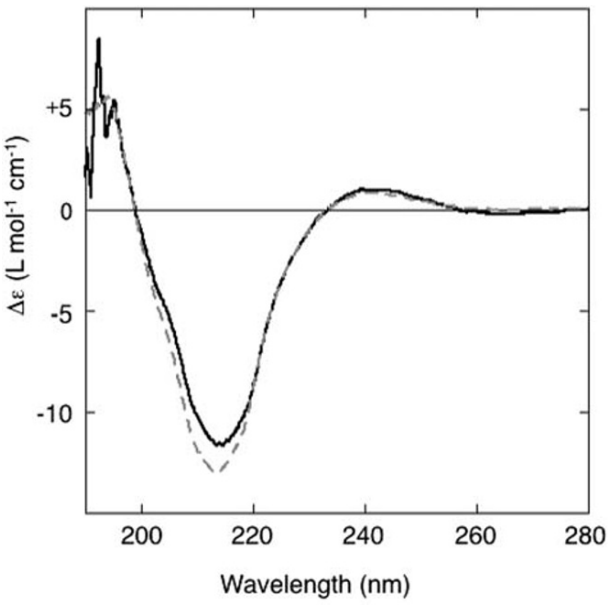


FIGURE 4 Comparison of ECD spectra for dolapyrrolidones (compound **1**) in acetonitrile. The curves of the natural and synthetic (*R*)-D-**1** are shown by solid and gray dotted lines, respectively

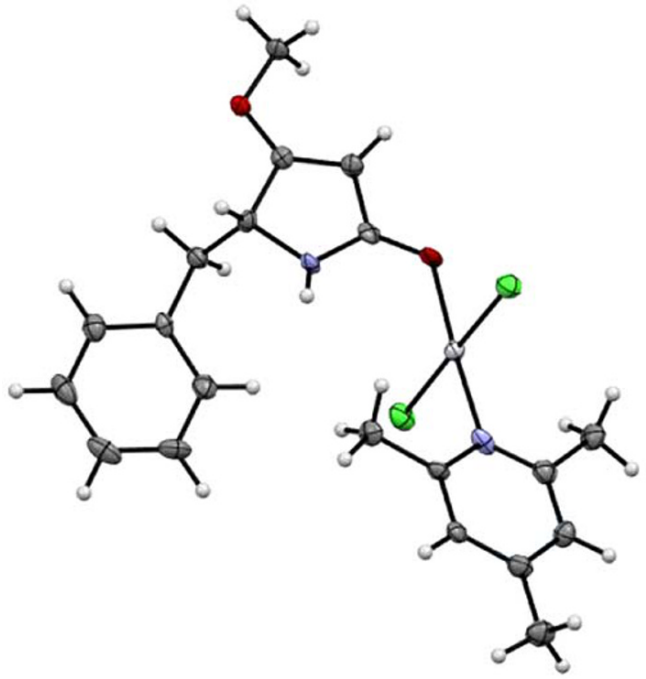
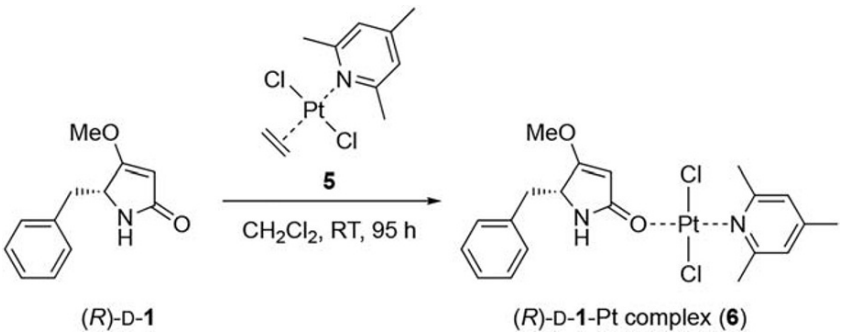


FIGURE 5 ORTEP drawing of synthetic dolapyrrolidone-Pt complex derived from (*R*)-D-**1** (ellipsoids set at 60% probability level)

The isolation of (*R*)-D-**1** as the natural product was not a good match with the fact that the reported Dpys as the substructure of natural peptides (dolastatin 15[7] and belamide A[13]) had been assigned as the natural L-amino acid. Accordingly, we have further confirmed our result by two more experiments, a chiral HPLC analysis (Figure 3) and an X-ray analysis (Figure 5) as a metal complex. We synthetically prepared all (*S*)-L-, (*R*)-D-, and *rac*-**1**, starting from the corresponding Boc-Phe-OH (by the method shown in Scheme 1). The obtained (*R*)-D-**1** was analyzed on a chiral HPLC in comparison with the natural Dpy. Detected with both ECD and UV detectors at 254 nm, the natural **1** exhibited the same retention time with (*R*)-D-**1**, though partial racemization was observed in both analyses. The natural **1** was analyzed again after keeping in a freezer for more than a year, exhibiting that a trace amount of racemization occurred (90.9% ee according to the chiral HPLC analysis, Figure 3), which might also have lowered the optical activity of natural **1**. The enantiomer separation was also observed with both *rac*- and (*S*)-L-**1** under the same condition. We also observed the strong solvent effect on the optical rotation: The synthetic (*R*)-D-**1** showed +76 in $CHCl_3$ and −36 in MeOH (c = 1.0 in both cases), which was a good match with the reported value.[11] This observation is also a good support for our assignment.

In addition, with the help of the synthetic (*R*)-D-**1**, the absolute stereochemistry of the natural **1** was confirmed by an X-ray analysis of Pt-complex derivative. Previously, part of the authors have developed new method for determination of absolute stereochemistry of chiral organic molecule by X-ray analysis with Pt-complexation process. For examples, absolute stereochemistry of planar-chiral cyclic alkenes were determined by X-ray analysis of crystalline π-coordinated Pt complex thereof, which was easily prepared by a simple mixing with *trans*-$PtCl_2$(2,4,6-trimethylpyridine)(ethylene) (**5**) in organic solvent.[14–17] Based on these, we envisioned that the Pt-complexation method would be applicable to the determination of the absolute stereochemistry of **1**. By a treatment of the synthetic sample of the same enantiomer of natural **1** with **5** in CH_2Cl_2 at RT for 95 h, crystalline Dpy-Pt complex **6** was obtained (Scheme 2). Interestingly enough, X-ray analysis of **6** revealed that Pt was σ-coordinated on the carbonyl oxygen (Figure 5), which is not similar with the case of planar-chiral alkenes to form π-coordinated Pt complexes. The anomalous dispersion effects in the diffraction measurement confirmed the absolute stereochemistry of the sample is (*R*)-D-form.

Natural dolapyrrolidone (Dpy) is presumably biosynthesized from phenylalanine via intramolecular condensation of *N*-acetylphenylalanine methyl ester[18] so that the absolute stereochemistry was expected to be the one derived from the natural L-phenylalanine or (*S*)-stereochemistry. The results of this study, however, indicate that the cyanobactrium *M.producens* may either utilize or



SCHEME 2 Preparation of (*R*)-D-**1**-Pt complex (**6**)

produce D-phenylalanine. There have been several peptides containing D-amino acids.[19] The role of D-amino acid in peptide has been explained to be (1) stabilization of specific conformation, (2) diversification of biological activity, and (3) prolongation of peptide. In some cases, introducing D-amino acids into a natural peptide is achieved by posttranslational modification. Other cases include the pathway via nonribosomal peptide synthetases (NRPSs),[20] a large assembly of multienzyme machineries, which occasionally adopt D-amino acids or their derivatives. The observed production of D-amino acid derivative in this study is also likely to follow a path similar to the latter, but the mechanism of this inversion is not obvious. Neither relevant peptides nor derivatives were detected in the above-mentioned analysis of the EtOAc extract by LC–MS. The absolute stereochemistry of the reported Dpy substructure are (*S*)-L-form, which was derived by chiral HPLC analysis after degradation for caldoramide[10] and belamide A.[13] For dolastatin 15[7] and smenamides A and B,[9,21] it was indirectly supported by the potent bioactivity of synthetic samples, in which (*S*)-L stereochemistry was presumed. The stereochemistry of Dpy substructure in mycapolyols A–F[8] has not seemed clearly determined. To gain some additional understandings, both the synthetic (*S*)-L- and (*R*)-D-**1** (as 10-μg/ml solution) were subjected to diatom growth inhibition[22] and cytotoxicity[23] tests, but no significant activity was observed.

4 | CONCLUSION

In summary, we have isolated natural dolapyrrolidone **1** from the cyanobacterium *M. producens* as the single compound for the first time. Compound **1** had been known as the substructure that is common among several bioactive natural peptides, in which the absolute stereochemistry was assigned as (*S*)-L. In this paper, however, the absolute stereochemistry of the isolated natural **1**, which was determined by the total synthesis, was (*R*)-D-form, the opposite to that of the known substructure. This surprising result was determined by chiral HPLC analysis by comparing the retention times of both the natural and synthetic enantiomers. Moreover, the absolute stereochemistry of natural **1** was separately reconfirmed by X-ray analysis of the Pt-complex derivative.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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