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# Isolation and total syntheses of two new alkaloids, dubiusamines-A, and -B, from *Pandanus dubius*

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ABSTRACT

Chemical investigation of the crude base of *Pandanus dubius* led to the isolation of two new alkaloids, dubiusamine-A (**1**) and dubiusamine-B (**2**). The structures of the two alkaloids including their absolute configuration were unambiguously confirmed by 1D and 2D NMR analysis and asymmetric total synthesis.



## 1. Introduction

The genus *Pandanus* (family Pandanaceae) comprises approximately 700 species distributed in tropical and sub-tropical regions. Several *Pandanus* species are used as medicinal plants for treating leprosy, rheumatism, epilepsy, and sore throat.[1] The isolation of alkaloids with a novel C9–N–C9 unit from *Pandanus amaryllifolius* had impelled interest in other *Pandanus* plants that may also contain alkaloids. To date, 16 alkaloids were identified from the genus *Pandanus*. As part of our research on novel biologically active alkaloids from the genus *Pandanus*,[2] we conducted a phytochemical analysis on *Pandanus dubius*. In this paper, we report the isolation of dubiusamine-A (**1**) and dubiusamine-B (**2**) from the crude base of *P. dubius* (Fig. 1), their structure elucidation by means of spectroscopic analysis, and their asymmetric total synthesis to determine their absolute configuration.

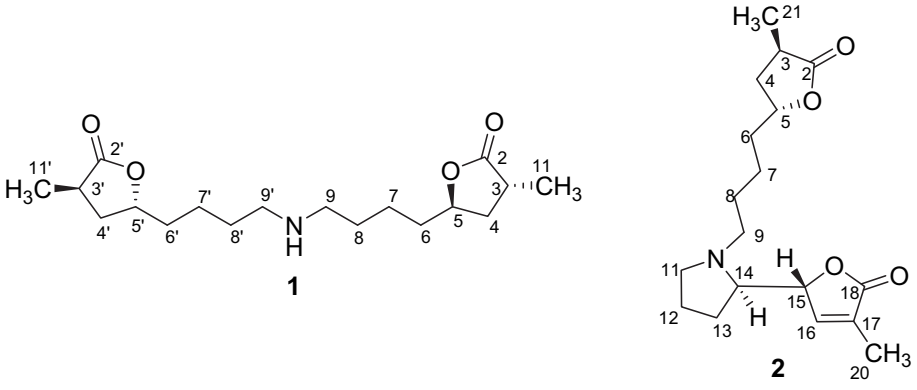


**Figure 1.** Structures of new alkaloids from *P. dubius*.

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## 2. Results and discussion

The crude base that was obtained using the conventional acid-base extraction procedure from a MeOH extract of *P. dubius* was separated by extensive column chromatography to obtain two new alkaloids, dubiusamine-A (**1**) and dubiusamine-B (**2**). In addition, seven known alkaloids, including pandamarilactonines-A,[2b] -B,[2b] and -C,[2c] pandamarilactones-1,[3] -31[3] and -32,[3] and pandamarilactam-3y,[4] were isolated. The known alkaloids were identified by comparing their spectroscopic data (NMR and MS) with reported values. The structures of **1** and **2** were unambiguously identified using spectroscopic and synthetic methods.

Dubiusamine-A (**1**) was obtained as an optically active {$[\alpha]_D^{20}$ +29.5 (*c* 0.07, $CHCl_3$)} amorphous solid in 0.12% yield (based on the crude alkaloid fraction). The molecular formula was established as $C_{18}H_{31}NO_4$ using HR-FABMS with its protonated molecular ion peak $[M+H]^+$ at *m*/*z* 326.2341 (calcd for $C_{18}H_{32}NO_4$, 326.2331). The $^{13}C$ NMR spectrum revealed the presence of nine carbons, symptomatic of a symmetrical structure. Through $^{13}C$ NMR and DEPT experiments, a lactone carbonyl ($\delta$ 180.1), an oxygenated methine ($\delta$ 78.3), a methylene associated to a nitrogen atom ($\delta$ 49.7), and further upfield methylenes, methine, and methyl were detected. The IR absorption band at 1759 $cm^{-1}$ validated the presence of a carbonyl of a lactone moiety. The $^1H$ NMR spectrum showed a total count of 15 hydrogen atoms in the molecule. Supported by $^1H$–$^1H$ COSY data (Fig. 2), a 3,5-disubstituted γ-butyrolactone was identified via correlations of protons associated to the alpha going to the gamma carbons. The linkage of the methyl group at C-3 with the *n*-butyl group at C-5 was also observed by further examination of the COSY spectrum. The gross structure of **1** was established by linking two units of the nine carbon segment to a nitrogen atom. This was corroborated by the chemical shift of the terminal carbon ($\delta$ 49.7, C-9, C-9′) of the butyl moiety. The HMBC experiment further supported the proposed structure. Long-range couplings were observed between the doublet methyl protons (H-11) and C-2, C-3, and C-4, and between the H-4 protons and C-2, C-3, and C-5. HMBC cross-peaks were also observed between the H-9 protons and C-7 and C-8 carbons.

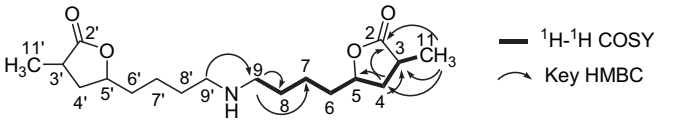
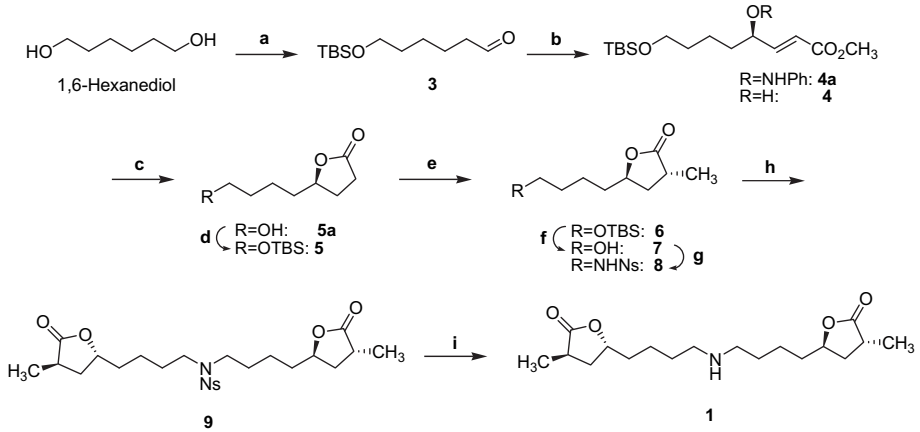



**Figure 2.** COSY and selected HMBC correlations of **1**.

Examination of the structure of **1** revealed the presence of two stereocenters — one at C-3 and the other at C-5. Detailed analysis of both $^1H$ and $^{13}C$ NMR spectra showed the occurrence of an inseparable 5:1 diastereomeric mixture. In particular, additional minor signals at $\delta$ 4.34 and 2.49 ($^1H$) and $\delta$ 37.3, 35.9, and 15.1 ($^{13}C$) were observed in the NMR spectra. To determine the stereochemical relationship of the substituents at C-3 and C-5, differential NOE experiment was carried out. Irradiation of the proton at $\delta$ 4.51 (H-5, H-5′) led to a clear peak enhancement of the methyl protons at $\delta$ 1.28 (H-11, H-11′). Hence, an *anti* relationship between the C-3 and C-5 methine protons is disclosed for the major diastereomer and a *syn* relationship is found[5] for the minor diastereomer. Although natural product **1** was obtained as a diastereomeric mixture, the total synthesis allowed for its isolation and characterization in pure form.

To unambiguously confirm the structure including its absolute configuration, the asymmetric total synthesis of **1** was conducted (Scheme 1). The synthesis started with the monoprotection of 1,6-hexanediol with TBSCl[6] and the oxidation of the remaining alcohol to give aldehyde **3**.[7] A three-step reaction that included the proline-mediated α-aminoxylation[8] of aldehyde **3** followed by the Horner–Emmons reaction and the O–N bond cleavage utilizing $CuSO_4$ in MeOH[9] afforded highly enantioselective ester **4** in 77% yield (over three steps) and 99% ee as determined by chiral HPLC analysis.[10] Based on the well-established mechanistic consideration for proline-catalyzed aldehyde α-aminoxylation,[8,9,11] the stereochemistry of the chiral center (C-4) in **4** was concluded to be *R*. Corresponding γ-butyrolactone **5** was furnished by subsequent hydrogenation using 10% Pd/C in MeOH. Treatment of **5** with LHMDS and MeI allowed the diastereoselective α-methylation of the lactone ring to give **6** in a 7:1 (*anti*/*syn*) ratio.[12] The removal of the TBS group in **6** was easily achieved using camphorsulfonic acid in MeOH at 0 °C to afford alcohol **7** in quantitative yield. The Mitsunobu reaction[13] employing a mixture of alcohol **7**, 2-nitrobenzenesulfonamide, $PPh_3$, and DEAD in THF/toluene gave, in 71% yield, corresponding nosyl-protected amine **8**. This compound was again treated with $PPh_3$ and DEAD in the presence of alcohol **7** using toluene as solvent to provide nosyl-protected symmetrical amide **9**. To complete the synthesis of **1**, deprotection of the nosyl group in amide **9** was accomplished with $K_2CO_3$ and PhSH. The product was carefully purified using silica gel chromatography to afford diastereomerically pure **1** {$[\alpha]_D^{23}$ +61.2 (*c* 0.05, $CHCl_3$)}. The spectroscopic data (IR, $^1H$ and $^{13}C$ NMR, HRMS) of synthetic **1** were identical to those of the natural product, thereby establishing the structure including the absolute configuration (3*R*, 5*R*) of dubiusamine-A.




**Scheme 1.** Synthetic route for dubiusamine-A. Reagents and conditions: (a) (i) TBSCl, NaH, THF, rt, 3 h; (ii) $SO_3$-pyridine complex, DMSO, $Et_3N$, $CH_2Cl_2$, 0 °C to rt, 3 h, 80% in two steps; (b) (i) L-Proline, Nitrosobenzene, $CH_3CN$, −20 °C, 20 h; (ii) Methyl diethyl phosphonoacetate, NaH, THF, 0 °C, 1 h; (iii) $CuSO_4$, MeOH, rt, 15 h, 77% in three steps; (c) 10% Pd/C, MeOH, $H_2$, rt, 45 h; (d) TBSCl, Imidazole, $CH_2Cl_2$, rt, 13 h, 96% in two steps; (e) LHMDS, MeI, THF, −78 °C, 6 h, 67% (dr 7:1); (f) CSA, MeOH, 0 °C, 1 h, quant.; (g) $NsNH_2$, $PPh_3$, DEAD,THF/Toluene, rt, 1 h, 71%; (h) Compound **7**, $PPh_3$, DEAD, Toluene, 60 °C, 1 h, 80%; (i) $K_2CO_3$, PhSH, DMF/$CH_3CN$, rt, 3 h, 96%.

Dubiusamine-B (**2**) was obtained as an optically active, amorphous solid {$[\alpha]_D^{18}$ +13.6 (*c* 0.025, $CHCl_3$)}. In its HR-FABMS spectrum, the protonated molecular ion peak $[M+H]^+$ at *m*/*z* 322.2028 (calcd for $C_{18}H_{28}NO_4$, 322.2018) agreed well with the molecular formula $C_{18}H_{27}NO_4$. $^1H$, $^{13}C$ (Table 1) and DEPT NMR spectra indicated two lactone carbonyls, five methines (two oxygen-bearing, one nitrogen-bearing and one $sp^2$), eight methylenes (two nitrogen-bearing), two methyls, and one olefinic quaternary carbon. The observed $^1H$ and $^{13}C$ NMR signals at {$\delta_H$ 7.06 (m, H-16), 4.81 (ddd, *J*=5.0, 1.8, 1.6, H-15), 1.94 (d, *J*=0.4, H-20); $\delta_C$ 174.4 (C-18), 146.9 (C-16), 131.3 (C-17), 83.7 (C-15), 10.8 (C-20)} and at {$\delta_H$ 2.80 (ddd, *J*=8.0, 5.5, 5.0, H-14); $\delta_C$ 54.3 (C-11), 25.9 (C-12), 23.9 (C-13), 65.4 (C-14)} are characteristic of a pyrrolidinyl-α-methyl-α,β-unsaturated γ-lactone moiety. This is a unique structural feature of known alkaloids pandamarilactonines-A and -C having a *threo* configuration at C-14 and C-15 positions.[2b,c] The structure of **2** was further concluded on the basis of $^1H$–$^1H$ COSY, HMQC, and HMBC spectroscopic analysis (Fig. 3). Both $^1H$ and $^{13}C$ NMR signals for upper portion of **2** having the 3,5-disubstituted γ-butyrolactone are coherent to those of **1**. In the HMBC spectrum, correlations in the pyrrolidinyl-α-methyl-α,β-unsaturated γ-lactone between H-14 and C-9/C-16; H-9 and C-11; H-15 and C-16; H-16 and C-18/C-20; and H-20 and C-17/C-18 were observed. Thus, the gross structure of dubiusamine-B was determined as in **2**.

**Table 1**
$^1H$ and $^{13}C$ NMR data for **2** in $CDCl_3$

| Position | $\delta_H$ (500 MHz) | $\delta_C$ (125 MHz) |
|---|---|---|
| 2 | | 180.0 |
| 3 | 2.70, m | 34.0 |
| 4 | 2.03, ddd (10.0, 7.2, 6.6)<br>2.12, ddd (10.5, 7.0, 4.0) | 35.5[a] |
| 5 | 4.51, m | 78.3 |
| 6 | 1.70–1.80, 2H, overlapped | 35.4[a] |
| 7 | 1.46–1.63, 2H, overlapped | 23.2 |
| 8 | 1.46–1.63, 2H, overlapped | 28.7 |
| 9 | 2.87, ddd (10.8, 7.9, 7.5)<br>2.40, ddd (11.0, 7.5, 6.0) | 55.6 |
| 11 | 3.12, dd (7.5, 6.5)<br>2.20, m | 54.3 |
| 12 | 1.70–1.80, overlapped<br>1.46–1.63, overlapped | 25.9 |
| 13 | 1.70–1.80, 2H, overlapped | 23.9 |
| 14 | 2.80, ddd (8.0, 5.5, 5.0) | 65.4 |
| 15 | 4.81, ddd (5.0, 1.8, 1.6) | 83.7 |
| 16 | 7.06, m | 146.9 |
| 17 | | 131.3 |
| 18 | | 174.4 |
| 20 | 1.94, 3H, d (0.4) | 10.8 |
| 21 | 1.28, 3H, d (7.5) | 15.9 |

[a] Interchangeable.

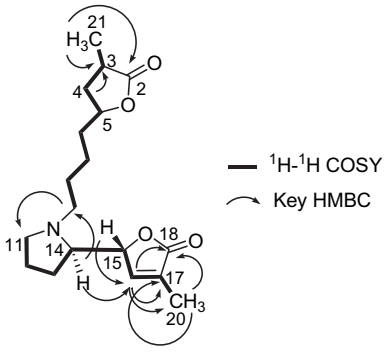



**Figure 3.** COSY and selected HMBC correlations of **2**.

To confirm the structure and establish the absolute stereochemistry of **2**, its total synthesis was attempted. As the absolute configuration at C-14 and C-15 in natural pandamarilactonine-A has already been demonstrated to be *R* and *R*,[2f] respectively, we have decided to use D-prolinol as our chiral starting material (Scheme 2). D-Prolinol was converted into known aldehyde **11**[2f] in two steps, and this was followed by vinylation of the aldehyde group to obtain diastereomeric adducts **12** in a 1:2 (i.e., less polar/more polar) ratio. After chromatographic separation of the diastereomers, their absolute configuration was determined by converting them into their oxazolidinone derivatives,[14] **16a** and **16b** (Scheme 3). Measurement of the coupling constants ($J_{H1,7a}$) and NOE crosspeak observation[15] between H-7a and H-1 for both **16a** and **16b** clearly indicated a (2′*R*, 1*S*, *erythro*) configuration for the less polar adduct and (2′*R*, 1*R*, *threo*) configuration for the more polar adduct. Removal of the Cbz group in the more polar adduct using TMSI in $CH_3CN$ afforded amino alcohol **13**. Condensation of **13** with the γ-butyrolactone iodide **10** having a 3*R*, 5*R* configuration in the presence of $Ag_2CO_3$ in $CH_3CN$ led to the formation of intermediate adduct **14**. Esterification of the hydroxyl group in **14** with methacrylic anhydride in the presence of $Et_3N$ and DMAP in $CH_2Cl_2$ easily gave ester **15**. The synthesis of **2** was completed by employing Grubbs second-generation catalyst in the presence of *p*-TsOH under reflux.[16] The spectroscopic data ($^1H$ NMR, $^{13}C$ NMR) including the high-resolution mass spectrometric and optical rotation {$[\alpha]_D^{17}$ +22 (*c* 0.30, $CHCl_3$)} data of the synthetic material, were in excellent agreement with those of the natural product. Thus, the structure including the absolute configuration (3*R*, 5*R*, 14*R*, 15*R*) of **2** was established.

## 3. Experimental section

### 3.1. General experimental procedures

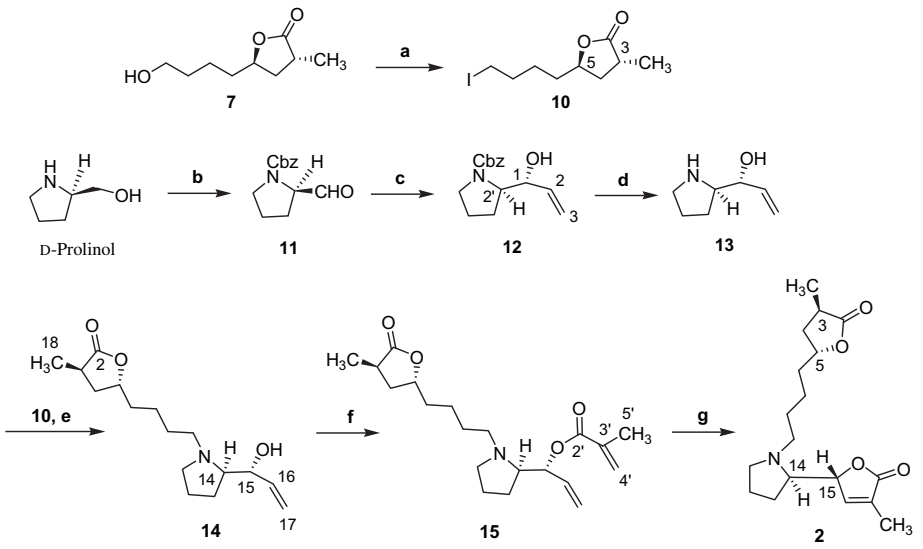
Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on JASCO FTIR-230 spectrophotometer. Low- and high-resolution FABMS were recorded on JEOL JMS-HX110 or JEOL JMS-AX500 mass spectrometer. HR-ESIMS were recorded on a Thermo Fisher Scientific Exactive spectrometer. NMR spectra were recorded on JEOL JNM A-500 and JEOL JNM ECP400 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants, in hertz. Kieselgel 60 [Merck, 70–230 mesh (for open column chromatography)], Silica gel 60 N [Kanto Chemical, 40–50 μm (for flash column chromatography)], Chromatorex NH [Fuji Silysia Chemical, (100–200 mesh), and $Al_2O_3$ 90 (Merck, Basic, Activity II–III) were used for column chromatography. Medium-pressure liquid chromatography was carried out on a silica gel prepacked column CPS-HS-221-05 (Kusano Kagakukikai).

### 3.2. Plant material

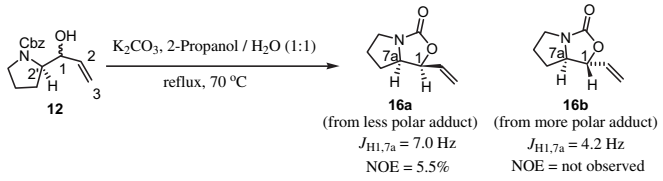
The fresh leaves of *P. dubius* were collected at PICC, Manila, Philippines, and identified by Assistant Professor Rosie Madulid, Department of Biological Sciences, College of Science, University of Santo Tomas. A voucher specimen (USTH-4846) was deposited at the Plant Sciences Herbarium, Research Center for the Natural Sciences, University of Santo Tomas.

### 3.3. Extraction and isolation

Air-dried and ground leaves of *P. dubius* (4.0 kg) were extracted with 5 L of MeOH (3×24 h) at room temperature and filtered. The combined filtrates were concentrated in vacuo to obtain the crude alcoholic extract (370 g). A portion of the crude extract (155 g) was partitioned between EtOAc and 1 N HCl. The aqueous layer was basified with $Na_2CO_3$ (pH 9–10) and exhaustively extracted with 5%




**Scheme 2.** Synthetic route for dubiusamine-B. Reagents and conditions: (a) $I_2$, $PPh_3$, Imidazole, $CH_2Cl_2$, rt, 3.5 h, 96%; (b) CbzCl, $K_2CO_3$, $CH_3CN$, −20 °C, 4 h; then $SO_3$–pyridine complex, DMSO, $Et_3N$, $CH_2Cl_2$, 0 °C, 3.5 h, 92% in two steps; (c)Vinyl magnesium bromide, THF, 0 °C, 3 h, 73% (dr 2:1); (d) TMSI, $CH_3CN$, −15 °C to 0 °C, 30 min 53%; (e) **10**, $Ag_2CO_3$, $CH_3CN$, rt, 45 h, 65%; (f) Methacrylic anhydride, $Et_3N$, DMAP, $CH_2Cl_2$, rt, 4 h, 71%; (g) *p*-TsOH, Grubbs second-generation catalyst, $CH_2Cl_2$, reflux, 19 h, 61%.




**Scheme 3.** Determination of absolute configuration.

MeOH/$CHCl_3$. The organic layer was dried with anhydrous $MgSO_4$ and evaporated to afford the crude alkaloidal fraction (2.1 g). A portion of the base (2 g) was subjected to silica gel flash column chromatography using $CHCl_3$/MeOH gradient to yield nine fractions: F1 (48.1 mg), $CHCl_3$; F2 (103.7 mg), 1% MeOH/$CHCl_3$; F3 (119.0 mg), 1–3% MeOH/$CHCl_3$; F4 (121.0 mg), 3–5% MeOH/$CHCl_3$; F5 (266.9 mg), 5–10% MeOH/$CHCl_3$; F6 (408.0 mg), 10–15% MeOH/$CHCl_3$; F7 (148.8 mg), 15–20% MeOH/$CHCl_3$; F8 (299.2 mg), 20–50% MeOH/$CHCl_3$; F9 (92.4 mg), 50% MeOH/$CHCl_3$/MeOH. F2 was subjected to silica gel open column chromatography (twice, 5% MeOH/$CHCl_3$) and MPLC (2% EtOH/$CHCl_3$) to afford pandamarilactone-1 (1.8 mg). F3 was subjected to gradient $CHCl_3$/MeOH silica gel open column chromatography to obtain sub-fractions F3A–F3D. Sub-fraction F3A was purified by repeated silica gel open column chromatography ($CHCl_3$/MeOH gradient) to afford pandamari lactonine-A (2.4 mg) and pandamarilactonine-B (1.1 mg). Sub-fraction F3B was purified by MPLC (50% EtOAc/hexane—2% EtOH/$CHCl_3$) to afford pandamarilactam-3y (3.1 mg). F4 was subjected to silica gel open column chromatography ($CHCl_3$/MeOH gradient), MPLC (twice, 3% MeOH/$CHCl_3$) and amino-silica gel column chromatography ($CHCl_3$) to afford pandamarilactone-31 (1.2 mg) and dubiusamine-B (**2**) (0.5 mg). Repeated silica gel open column chromatography of F5 ($CHCl_3$/MeOH gradient) and further purification by amino-silica gel column chromatography gave pandamarilactonine-A (1.1 mg), pandamarilactonine-C (0.8 mg), and pandamarilactone-32 (1.3 mg). Silica gel open column chromatography of F7 ($CHCl_3$/MeOH gradient) gave mixtures of pandamarilactonines-A and -B or pandamarilactonines-A and -C. Fraction F9 was purified by silica gel (15–30% MeOH/$CHCl_3$—MeOH) and amino-silica gel (thrice, 5% MeOH/$CHCl_3$) open column chromatography to give dubiusamine-A (**1**) (2.3 mg).

*3.3.1. Dubiusamine-A (**1**).* Amorphous solid; $[\alpha]_D^{20}$ +29.5 (*c* 0.07, $CHCl_3$); IR (ATR) $\nu_{max}$ 2936, 1759, 1160 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 4.51 (2H, dddd, *J*=13.0, 8.0, 8.0, 5.0 Hz, H-5, H-5′), 2.69 (2H, m, H-3, H-3′), 2.61 (4H, dd, *J*=6.8, 6.8 Hz, $H_2$-9, $H_2$-9′), 2.12 (2H, ddd, *J*=10.4, 6.6, 4.0 Hz, H-4b, H-4′b), 2.00 (2H, ddd, *J*=10.4, 6.0, 6.0 Hz, H-4a, H-4′a), 1.70 (2H, m, H-6, H-6′), 1.60–1.46 (10H, m, overlapped, H-6, H-6′, $H_2$-7, $H_2$-7′, $H_2$-8, $H_2$-8′), 1.28 (6H, d, *J*=7.0 Hz, $H_3$-11, $H_3$-11′); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 180.1 (C, C-2, C-2′), 78.3 (CH, C-5, C-5′), 49.7 ($CH_2$, C-9, C-9′), 35.5 ($CH_2$, C-4, C-4′), 35.4 ($CH_2$, C-6, C-6′), 34.0 (CH, C-3, C-3′), 29.7 ($CH_2$, C-8, C-8′), 23.3 ($CH_2$, C-7, C-7′), 15.9 ($CH_3$, C-11, C-11′); HR-FABMS: calcd for $C_{18}H_{32}NO_4$ $[M+H]^+$: 326.2331, found: 326.2341.

*3.3.2. Dubiusamine-B (**2**).* Amorphous solid; $[\alpha]_D^{18}$ +13.6 (*c* 0.025, $CHCl_3$); UV $\lambda_{max}$ 209, 243, 273 nm; $^1H$ and $^{13}C$ NMR data, see Table 1; FABMS (NBA) *m*/*z* 322 $[M+H]^+$; HR-FABMS: calcd for $C_{18}H_{28}NO_4$, $[M+H]^+$: 322.2018, found: 322.2028.

### 3.4. Preparation and characterization of the new compounds

*3.4.1. Ester (**4**).* To a stirred solution of **3** (100.8 mg, 0.440 mmol) and nitrosobenzene (94.0 mg, 0.880 mmol, 2 equiv) in $CH_3CN$ (1.1 mL) was added L-proline (20.2 mg, 0.180 mmol, 0.4 equiv) at −20 °C and the mixture was stirred for 20 h. In another reaction flask, methyl diethyl phosphonoacetate (0.16 mL, 0.880 mmol, 2 equiv) was added dropwise to a stirred solution of NaH (35.2 mg, 0.880 mmol, 2 equiv) in THF (1 mL) in an ice bath. The mixture was stirred at room temperature for 45 min before transferring to the above solution of **3** at 0 °C via a cannula. The resulting mixture was stirred for 1 h at 0 °C and then quenched with cold saturated $NH_4Cl$, and the resulting aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by silica gel flash column chromatography (20% EtOAc/hexane) afforded **4a** (149.0 mg, 86% yield) as an orange-amorphous solid. To a stirred solution of **4a** (149.0 mg, 0.380 mmol) in MeOH (1.9 mL) was added $CuSO_4$ (18.1 mg, 0.114 mmol, 0.3 equiv) at room temperature and the mixture was stirred for 15 h. The reaction mixture was quenched with saturated $NH_4Cl$ and the aqueous layer was extracted three times with $CHCl_3$. The combined $CHCl_3$ layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Title compound **4** was obtained after silica gel

flash column chromatography (20% EtOAc/hexane) in 89% yield (102.0 mg, 77% yield over three steps) as an orange-amorphous solid; $[\alpha]_D^{24}$ −44.1(*c* 0.3, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 206 nm; IR (ATR) $\nu_{max}$ 3437, 2929, 2857, 1725, 1659, 1471, 1253 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 6.95 (1H, dd, *J*=15.6, 4.8 Hz, H-3), 6.05 (1H, dd, *J*=16.0, 2.0 Hz, H-2), 4.32 (1H, m, H-4), 3.74 (3H, s, $OCH_3$), 3.62 (2H, dd, *J*=6.0, 6.0 Hz, $H_2$-8), 1.63–1.47 (6H, m, $H_2$-5, $H_2$-6, $H_2$-7), 0.90 (9H, s, $C(CH_3)_3$), 0.05 (6H, s, $Si(CH_3)_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 166.9 (C, C-1), 150.4 (CH, C-3), 119.7 (CH, C-2), 71.0 (CH, C-4), 70.0 ($CH_2$, C-8), 51.6 ($CH_3$, $OCH_3$), 36.3 ($CH_2$, C-7), 32.3 ($CH_2$, C-5), 25.9 ($CH_3$, $C(CH_3)_3$), 21.6 ($CH_2$, C-6), 18.3 (C, $C(CH_3)_3$), −5.5 ($CH_3$, $Si(CH_3)_2$); HRESIMS: calcd for $C_{15}H_{31}O_4Si$, $[M+H]^+$: 303.1986, found: 303.1979.

*3.4.2. γ-Butyrolactone (**5**).* To a stirred solution of **4** (102.0 mg, 0.337 mmol) in MeOH (6.7 mL) was added 10% Pd/C (50.0 mg). The reaction mixture was stirred under hydrogen atmosphere (balloon) for 45 h and filtered using a pad of Celite (EtOAc). The filtrate was concentrated under reduced pressure. The crude material was subjected to silica gel flash column chromatography (EtOAc) to afford **5a** (54.1 mg) as colorless oil in quantifiable yield. To a solution of **5a** (54.1 mg, 0.342 mmol) in $CH_2Cl_2$ (0.68 mL) was added imidazole (46.6 mg, 0.684 mmol, 2 equiv) and the resulting mixture was stirred in an ice bath. TBSCl (62.0 mg, 0.410 mmol, 1.2 equiv) was added and the mixture was stirred for an additional 1 h in an ice bath. The temperature of the mixture was allowed to return to room temperature and stirring was commenced for 13 h. The reaction mixture was quenched with saturated $NH_4Cl$ and the aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel flash column chromatography (30% EtOAc/hexane) afforded title compound **5** as a colorless oil in 96% yield over two steps (89.6 mg); $[\alpha]_D^{22}$ −35.4 (*c* 0.3, $CHCl_3$); IR (ATR) $\nu_{max}$ 2922, 2858, 1750, 1213 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 4.50 (1H, m, H-5), 3.62 (2H, dd, *J*=6.2, 6.2 Hz, $H_2$-9), 2.33 (1H, ddd, *J*=13.6, 6.8, 6.8 Hz, H-4), 2.53 (2H, m, $H_2$-3), 1.91–1.49 (7H, m, H-4, $H_2$-6, $H_2$-7, $H_2$-8), 0.90 (9H, s, $C(CH_3)_3$), 0.05 (6H, s, $Si(CH_3)_2$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 177.2 (C, C-2), 81.0 (CH, C-5), 62.8 ($CH_2$, C-9), 35.3 ($CH_2$, C-6), 32.4 ($CH_2$, C-8), 28.8 ($CH_2$, C-3), 28.0 ($CH_2$, C-4), 25.9 ($CH_3$, $C(CH_3)_3$), 21.7 ($CH_2$, C-7), 18.3 (C, $C(CH_3)_3$), −5.3 ($CH_3$, $Si(CH_3)_2$); HRESIMS: calcd for $C_{14}H_{29}O_3Si$ $[M+H]^+$: 273.1886, found: 273.1895.

*3.4.3. Alcohol (**7**).* A solution of **5** (124.0 mg, 0.455 mmol) in THF (1.0 mL) was added to lithium bis(trimethylsilyl)amide (1.0 M in THF, 0.5 mL, 0.500 mmol, 1.1 equiv) at −78 °C via a cannula. The mixture was stirred for 1 h at −78 °C and methyl iodide (0.426 mL, 6.82 mmol, 15 equiv) was added dropwise to it. The mixture was stirred for another 5 h before it was allowed to warm at −20 °C and quenched with 1 N HCl. The resulting mixture was extracted three times with $CHCl_3$ and the combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Compound **6** was obtained after silica gel flash column chromatography (20% EtOAc/hexane) as a colorless oil in 67% yield (87.0 mg, dr 7:1, *anti*/*syn*; FABMS (NBA): *m*/*z* 287 $[M+H]^+$). To a stirred solution of **6** (56.0 mg, 0.200 mmol) in MeOH (2.0 mL) was added camphorsulfonic acid (CSA, 19.1 mg, 0.040 mmol, 0.2 equiv) at 0 °C. After 1 h, the reaction mixture was quenched with saturated $NaHCO_3$ and the aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure. Purification by silica gel flash column chromatography (20% MeOH/$CHCl_3$) afforded title compound **7** as a colorless oil in quantifiable yield; $[\alpha]_D^{22}$ +40 (*c* 0.01, $CHCl_3$); IR (ATR) $\nu_{max}$ 3419, 2934, 2866, 1752, 1455, 1185 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 4.51 (1H, dddd, *J*=13.0, 8.0, 8.0, 5.5 Hz, H-5), 3.67 (2H, dd, *J*=6.3, 6.3 Hz, $H_2$-9), 2.69 (1H, m, H-3), 2.12 (1H, ddd, *J*=10.8, 7.0, 6.9 Hz, H-4a), 2.00 (1H, ddd, *J*=10.7, 7.0, 7.0 Hz, H-4b), 1.76–1.43 (6H, m, $H_2$-6, $H_2$-7, $H_2$-8), 1.28 (3H, d, *J*=7.0 Hz, $H_3$-10); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 180.1 (C, C-2), 78.3 (CH, C-5), 62.5 ($CH_2$, C-9), 35.4 ($CH_2$, C-4), 35.2 ($CH_2$, C-6), 34.0 (CH, C-3), 32.2 ($CH_2$, C-8), 21.7 ($CH_2$, C-7), 15.9 ($CH_3$, C-10); HRESIMS: calcd for $C_9H_{16}O_3Na$ $[M+Na]^+$: 195.0992, found: 195.0992.

*3.4.4. Nosyl-protected amine (**8**).* To a stirred solution of **7** (15.0 mg, 0.087 mmol) in THF (0.4 mL) and toluene (0.6 mL) were added triphenylphosphine (29.7 mg, 0.113 mmol, 1.3 equiv) and 2-nitrobenzenesulfonamide (44.0 mg, 0.218 mmol, 2.5 equiv), respectively, at room temperature. The solution was allowed to cool in an ice bath and DEAD (40% in toluene, 49 μL, 0.113 mmol, 1.3 equiv) was added dropwise. The reaction mixture was stirred at room temperature for 1 h. The mixture was filtered using a Celite pad and the filtrate was concentrated under reduced pressure. Title compound **8** was obtained after purification using MPLC (50% EtOAc/hexane) and silica gel flash column chromatography (hexane/EtOAc/$CHCl_3$=1/0.5/0.5) as a light-yellow oil in 71% yield (22.1 mg); $[\alpha]_D^{23}$ +31.8 (*c* 0.033, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 204, 221 (sh) nm; IR (ATR) $\nu_{max}$ 2935, 2866, 1755, 1537, 1338, 1161 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.14 (1H, m, Nosyl group), 7.88 (1H, m, Nosyl group), 7.76 (2H, m, Nosyl group), 5.27 (1H, dd, *J*=5.8, 5.8 Hz, N*H*), 4.46 (1H, m, H-5), 3.10 (2H, q, *J*=6.4, 2.0 Hz, $H_2$-9), 2.65 (1H, m, H-3), 2.07 (1H, ddd, *J*=12.4, 8.8, 5.6 Hz, H-4a), 1.99 (1H, ddd, *J*=12.2, 8.8, 5.6 Hz, H-4b), 1.67–1.47 (6H, m, $H_2$-6, $H_2$-7, $H_2$-8), 1.27 (3H, d, *J*=7.2 Hz, $H_3$-10); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 179.8 (C, C-2), 148.1 (C), 133.7 (CH), 133.6 (C), 132.8 (CH), 131.1 (CH), 125.4 (CH) [Nosyl group], 77.9 (CH, C-5), 43.5 ($CH_2$, C-9), 35.4 ($CH_2$, C-6), 34.9 ($CH_2$, C-4), 34.0 (CH, C-3), 29.3 ($CH_2$, C-8), 22.5 ($CH_2$, C-7), 15.9 ($CH_3$, C-10); HRESIMS: calcd for $C_{15}H_{20}N_2O_6NaS$ $[M+Na]^+$: 379.0934, found: 379.0922.

*3.4.5. Nosyl-protected symmetrical amide (**9**).* To a stirred mixture of **8** (17.5 mg, 0.050 mmol), **7** (12.9 mg, 0.075 mmol, 1.5 equiv), and triphenylphosphine (17.0 mg, 0.065 mmol, 1.3 equiv) in toluene (0.32 mL) was added DEAD (40% in toluene, 28 μL, 0.065 mmol, 1.3 equiv) dropwise at 0 °C (ice bath). The mixture was heated at 60 °C for 1 h. After cooling, the reaction mixture was filtered using a Celite pad and the filtrate was concentrated under reduced pressure. The crude residue was purified by MPLC (90% EtOAc/hexane) to obtain title compound **9** as a light-yellow amorphous solid in 80% yield (20.5 mg); $[\alpha]_D^{22}$ +20.9 (*c* 0.02, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 206 nm; IR (ATR) $\nu_{max}$ 2937, 1759, 1542, 1455, 1159 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.00 (1H, m, Nosyl group), 7.71 (2H, m, Nosyl group), 7.62 (1H, m, Nosyl group), 4.46 (2H, m, H-5, H-5′), 3.28 (4H, m, $H_2$-9, $H_2$-9′), 2.67 (2H, m, H-3, H-3′), 2.07 (2H, ddd, *J*=12.4, 8.8, 5.6 Hz, H-4b, H-4b′), 1.99 (2H, ddd, *J*=12.2, 8.8, 5.6 Hz, H-4a, H-4a′), 1.65–1.38 (12H, m, $H_2$-6, $H_2$-6′, $H_2$-7, $H_2$-7′, $H_2$-8, $H_2$-8′), 1.27 (6H, d, *J*=7.2 Hz, $H_3$-10, $H_3$-10′); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 180.0 (C, C-2, C-2′), 148.0 (C), 133.5 (CH), 133.4 (C), 132.7 (CH), 130.7 (CH), 124.1 (CH) [Nosyl group], 78.0 (CH, C-5, C-5′), 47.1 ($CH_2$, C-9, C-9′), 35.4 ($CH_2$, C-6, C-6′), 34.8 ($CH_2$, C-4, C-4′), 33.9 (CH, C-3, C-3′), 27.8 ($CH_2$, C-8, C-8′), 22.5 ($CH_2$, C-7, C-7′), 15.9 ($CH_3$, C-10, C-10′); HRESIMS: calcd for $C_{24}H_{34}N_2O_8NaS$ $[M+Na]^+$: 533.1928, found: 533.1916.

*3.4.6. Synthetic dubiusamine-A (**1**).* To a stirred solution of **9** (20.0 mg, 0.039 mmol) in DMF (50 μL) and $CH_3CN$ (80 μL) were added $K_2CO_3$ (10.8 mg, 0.078 mmol, 2 equiv) and thiophenol (5.2 μL, 0.051 mmol, 1.3 equiv) and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtered using a Celite pad and the filtrate was evaporated under reduced pressure. The crude residue was purified by silica gel open column chromatography (5% MeOH*/$CHCl_3$, MeOH*=10% $NH_3$ in MeOH) to afford synthetic **1** in 96% yield (12.3 mg); $[\alpha]_D^{23}$ +61.2 (*c* 0.05, $CHCl_3$); HRESIMS: calcd for $C_{18}H_{32}NO_4$ $[M+H]^+$: 326.2326, found:

326.2317; IR, $^1$H, and $^{13}$C NMR data of synthetic **1** were identical with those of natural **1**.

*3.4.7. γ-Butyrolactone iodide (**10**).* To a stirred solution of **7** (16.0 mg, 0.093 mmol) in $CH_2Cl_2$ (4.7 mL) were added triphenylphosphine (44.0 mg, 0.170 mmol, 1.8 equiv) and imidazole (17.0 mg, 0.240 mmol, 2.6 equiv). The mixture was cooled in an ice bath and then $I_2$ (43 mg, 0.170 mmol, 1.8 equiv) was added. The mixture was stirred at room temperature for 3.5 h. Saturated aqueous $Na_2S_2O_3$ (4.7 mL) was added to the mixture and the mixture was further stirred for 15 min. The mixture was quenched with saturated $NaHCO_3$ and extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Title compound **10** was obtained after purification using MPLC (30% EtOAc/hexane) as a colorless oil in 96% yield (25.0 mg); $[\alpha]_D^{28}$ +72 (*c* 0.02, $CHCl_3$); IR (ATR) $\nu_{max}$ 1755 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 4.52 (1H, dddd, *J*=12.8, 7.6, 7.6, 4.8 Hz, H-5), 3.20 (2H, dd, *J*=7.0, 7.0 Hz, $H_2$-9), 2.70 (1H, m, H-3), 2.13 (1H, ddd, *J*=12.8, 9.0, 5.2 Hz, H-4a), 2.03 (1H, ddd, *J*=12.8, 7.6, 7.6 Hz, H-4b), 1.87 (2H, quin, *J*=7.2 Hz, $H_2$-8), 1.75–1.49 (4H, m, $H_2$-6, $H_2$-7), 1.29 (3H, d, *J*=7.2 Hz, $H_3$-11); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 179.9 (C, C-2), 78.0 (CH, C-5), 35.4 ($CH_2$, C-6), 34.3 ($CH_2$, C-4), 34.0 (CH, C-3), 32.9 ($CH_2$, C-8), 26.4 ($CH_2$, C-7), 15.9 ($CH_3$, C-11), 6.2 ($CH_2$, C-9); HRESIMS: calcd for $C_9H_{15}O_2INa$ $[M+Na]^+$: 305.0002, found: 305.0009.

*3.4.8. Adduct (**12**).* To a stirred solution of **11** (1.2 g, 5.15 mmol) in THF (10.3 mL) at 0 °C was added vinyl magnesium bromide (7.7 mL, 7.73 mmol, 1.5 equiv) and the reaction mixture was stirred for 3 h. The mixture was quenched with saturated $NH_4Cl$ and the resulting aqueous mixture was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Title compound **12** was obtained as a 2:1 diastereomeric mixture after silica gel flash column chromatography (40% EtOAc/hexane) in 73% yield (990.0 mg). Separation of the diastereomers was achieved using MPLC (20% EtOAc/hexane); Less polar diastereomer (*erythro*): $[\alpha]_D^{22}$ +56.2 (*c* 0.5, $CHCl_3$); $^1$H NMR (DMSO-$d_6$, VT 100, 400 MHz) $\delta$ 7.35–7.28 (5H, m, Ph of Cbz), 5.80 (1H, ddd, *J*=16.0, 10.8, 5.2 Hz, H-2), 5.19 (1H, dd, *J*=17.2, 2.0 Hz, H-3a), 5.10 (2H, s, $CH_2$ of Cbz), 5.05 (1H, dd, *J*=10.4, 1.8 Hz, H-3b), 4.63 (1H, d, *J*=5.2 Hz, O*H*), 4.38 (1H, br s, H-1), 3.79 (1H, m, H-2′), 3.46 (1H, m, H-5′a), 3.31 (1H, m, H-5′b), 1.81 (4H, m, $H_2$-3′, $H_2$-4′); $^{13}$C NMR (DMSO-$d_6$, VT 100, 150 MHz) $\delta$ 153.3 (C=O of Cbz), 136.2 (C), 127.2 (CH), 126.5 (CH), 126.3 (CH) [Ph of Cbz], 138.7 (CH, C-2), 113.1 ($CH_2$, C-3), 70.7 (CH, C-1), 64.8 ($CH_2$ of Cbz), 60.7 (CH, C-2′), 45.9 ($CH_2$, C-5′), 23.9 ($CH_2$, C-3′), 22.5 ($CH_2$, C-4′); More polar diastereomer (*threo*): $[\alpha]_D^{23}$ +64.9 (*c* 0.22, $CHCl_3$); IR (ATR) $\nu_{max}$ 3422, 2975, 1671, 1497, 1412 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, VT 100, 400 MHz) $\delta$ 7.35–7.28 (5H, m, Ph of Cbz), 5.80 (1H, ddd, *J*=16.4, 10.8, 5.2 Hz, H-2), 5.17 (1H, dd, *J*=17.2, 2.0 Hz, H-3a), 5.10 (2H, s, $CH_2$ of Cbz), 5.07 (1H, dd, *J*=10.4, 2.0 Hz, H-3b), 4.67 (1H, d, *J*=4.4 Hz, O*H*), 4.36 (1H, q, *J*=5.0 Hz, H-1), 3.93 (1H, m, H-2′), 3.46 (1H, m, H-5′a), 3.24 (1H, m, H-5′b), 1.79 (4H, m, $H_2$-3′, $H_2$-4′); $^{13}$C NMR (DMSO-$d_6$, VT 100, 150 MHz) $\delta$ 153.6 (C=O of Cbz), 136.2 (C), 127.2 (CH), 126.5 (CH), 126.3 (CH) [Ph of Cbz], 137.3 (CH, C-2), 114.0 ($CH_2$, C-3), 70.6 (CH, C-1), 64.9 ($CH_2$ of Cbz), 60.5 (CH, C-2′), 45.9 ($CH_2$, C-5′), 24.6 ($CH_2$, C-3′), 22.3 ($CH_2$, C-4′); HRESIMS: calcd for $C_{15}H_{19}NO_3Na$ $[M+Na]^+$: 284.1250, found: 284.1257.

*3.4.9. Amino alcohol (**13**).* To a stirred solution of **12** (163.0 mg, 0.700 mmol) in $CH_3CN$ (4.7 mL) at −15 °C was added TMSI (0.5 mL, 3.50 mmol, 5 equiv). The reaction mixture was stirred for 15 min at −15 °C and another 15 min at 0 °C. The mixture was quenched with MeOH and evaporated under reduced pressure. Purification by silica gel flash column chromatography and $Al_2O_3$ column chromatography (10% MeOH/$CHCl_3$) afforded title compound **13** as a colorless oil in 53% yield (47.0 mg); $[\alpha]_D^{23}$ −5.5 (*c* 0.29, $CHCl_3$); IR (ATR) $\nu_{max}$ 3316, 2966, 2875, 1631, 1536 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 5.90 (1H, ddd, *J*=17.0, 10.0, 6.8 Hz, H-2), 5.33 (1H, dd, *J*=17.0, 1.6 Hz, H-3a), 5.17 (1H, dd, *J*=10.0, 1.6 Hz, H-3b), 3.73 (1H, dd, *J*=9.5, 6.8 Hz, H-1), 3.11 (1H, ddd, *J*=8.0, 7.2, 7.2 Hz, H-2′), 2.97 (1H, m, H-5′a), 2.88 (1H, m, H-5′b), 1.85–1.56 (4H, m, $H_2$-3′, $H_2$-4′); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 135.4 (CH, C-2), 119.7 ($CH_2$, C-3), 72.1 (CH, C-1), 64.6 (CH, C-2′), 45.7 ($CH_2$, C-5′), 27.2 ($CH_2$, C-4′), 24.1 ($CH_2$, C-3′); HRESIMS: calcd for $C_7H_{14}NO$ $[M+H]^+$: 128.1070, found: 128.1076.

*3.4.10. Condensed adduct (**14**).* To a stirred solution of **13** (10.0 mg, 0.079 mmol) and **10** (27.0 mg, 0.095 mmol, 1.2 equiv) in $CH_3CN$ (1.0 mL) was added $Ag_2CO_3$ (50 wt % on Celite, 65.3 mg, 0.119 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 45 h. The heterogeneous mixture was then filtered using a Celite pad and the filtrate was concentrated under reduced pressure. Purification by silica gel open column chromatography (50% EtOAc/hexane to 5% MeOH/$CHCl_3$) afforded title compound **14** as a colorless oil in 65% yield (14.2 mg); $[\alpha]_D^{19}$ +56.4 (*c* 0.17, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 5.83 (1H, ddd, *J*=17.0, 10.0, 5.5 Hz, H-16), 5.31 (1H, dd, *J*=17.1, 1.5 Hz, H-17a), 5.13 (1H, dd, *J*=10.0, 1.5 Hz, H-17b), 4.50 (1H, dddd, *J*=13.0, 8.0, 8.0, 5.5 Hz, H-5), 3.73 (1H, dd, *J*=5.5, 5.5 Hz, H-15), 3.12 (1H, ddd, *J*=10.0, 6.5, 4.0 Hz, H-11a), 2.75 (1H, m, H-9a), 2.68 (2H, overlapped, H-3, H-14), 2.43 (1H, m, H-9b), 2.37 (1H, m, H-11b), 2.10 (1H, ddd, *J*=12.5, 8.5, 5.0 Hz, H-4a), 2.00 (1H, m, H-4b), 1.89 (1H, m, H-12a), 1.76 (2H, m, $H_2$-13), 1.72–1.51 (6H, m, $H_2$-6, H-7, $H_2$-8, H-12b), 1.38 (1H, m, H-7), 1.28 (3H, d, *J*=7.0 Hz, $H_3$-18); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 180.0 (C, C-2), 139.8 (CH, C-16), 115.2 ($CH_2$, C-17), 78.3 (CH, C-5), 74.2 (CH, C-15), 68.8 (CH, C-14), 57.1 ($CH_2$, C-9), 54.1 ($CH_2$, C-11), 35.4 ($CH_2$, C-4), 35.3 ($CH_2$, C-6), 34.0 (CH, C-3), 28.4 ($CH_2$, C-12), 28.2 ($CH_2$, C-8), 24.3 ($CH_2$, C-13), 23.0 ($CH_2$, C-7), 15.9 ($CH_3$, C-18); HRESIMS: calcd for $C_{16}H_{28}NO_3$ $[M+H]^+$: 282.2064, found: 282.2057.

*3.4.11. Ester (**15**).* To a stirred solution of **14** (11.4 mg, 0.041 mmol) in $CH_2Cl_2$ (0.41 mL) at 0 °C were added $Et_3N$ (17 μL, 0.123 mmol, 3 equiv) and DMAP (5.0 mg, 0.041 mmol, 1 equiv). Methacrylic anhydride (14 μL, 0.090 mmol, 2.2 equiv) was then added dropwise and the resulting mixture was stirred at room temperature for 4 h. The reaction was quenched with saturated $NH_4Cl$ and the aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification by silica gel column chromatography (3% MeOH/$CHCl_3$) afforded title compound **15** as a colorless oil in 71% yield (10.2 mg); $[\alpha]_D^{19}$ +16.4 (*c* 0.06, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 6.14 (1H, br s, H-4′a), 5.95 (1H, ddd, *J*=15.6, 10.0, 4.8 Hz, H-16), 5.57 (1H, br d, *J*=1.6 Hz, H-4′b), 5.35 (1H, dd, *J*=5.2, 5.2 Hz, H-15), 5.26 (1H, dd, *J*=15.6, 1.6 Hz, H-17a), 5.22 (1H, dd, *J*=9.8, 1.6 Hz, H-17b), 4.51 (1H, dddd, *J*=13.0, 8.0, 8.0, 5.5 Hz, H-5), 3.09 (1H, ddd, *J*=8.8, 6.0, 2.8 Hz, H-11a), 2.83 (1H, m, H-9a), 2.70 (2H, overlapped, H-3, H-14), 2.39 (1H, m, H-9b), 2.16 (1H, m, H-11b), 2.09 (1H, ddd, *J*=13.0, 8.8, 4.4 Hz, H-4a), 2.00 (1H, m, H-4b), 1.96 (3H, dd, *J*=1.4, 1.0 Hz, $H_3$-5′), 1.75–1.47 (10H, m, $H_2$-6, $H_2$-7, $H_2$-8, $H_2$-12, $H_2$-13), 1.28 (3H, d, *J*=7.2 Hz, $H_3$-18); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 180.1 (C, C-2), 166.4 (C, C-2′), 136.5 (CH, C-16), 133.5 (C, C-3′), 125.4 ($CH_2$, C-4′), 116.8 ($CH_2$, C-17), 78.4 (CH, C-5), 76.0 (CH, C-15), 65.7 (CH, C-14), 55.6 ($CH_2$, C-9), 54.3 ($CH_2$, C-11), 35.4 ($CH_2$, C-4), 35.3 ($CH_2$, C-6), 34.0 (CH, C-3), 28.7 ($CH_2$, C-8), 26.0 ($CH_2$, C-12), 23.9 ($CH_2$, C-13), 23.2 ($CH_2$, C-7), 18.3 ($CH_3$, C-5′), 15.9 ($CH_3$, C-18); HRESIMS: calcd for $C_{20}H_{32}NO_4$ $[M+H]^+$: 350.2326, found: 350.2312.

*3.4.12. Synthetic dubiusamine-B (**2**).* A mixture of **15** (14.0 mg, 0.040 mmol) and *p*-TsOH (7.6 mg, 0.040 mmol, 1 equiv) was dissolved with $CH_2Cl_2$ (4.0 mL). The resulting mixture was refluxed for 30 min and Grubbs second-generation catalyst (5 mg, 6.0 μmol, 0.15 equiv) in 0.5 mL of $CH_2Cl_2$ was then added. The mixture was stirred under reflux for 19 h. The mixture was cooled to room temperature and quenched with 1 M NaOH, and the aqueous layer was extracted three times with 5% MeOH/$CHCl_3$. The combined

organic layers were washed with 1 M NaOH and then brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude residue was purified with amino-silica gel ($CHCl_3$), followed by MPLC (2% EtOH/$CHCl_3$) to give synthetic **2** in 61% yield (7.8 mg); $[\alpha]_D^{17}$ +22 (*c* 0.30, $CHCl_3$); HRESIMS calcd for $C_{18}H_{28}NO_4$ $[M+H]^+$: 322.2013, found: 322.2013; $^1H$ and $^{13}C$ NMR data of synthetic **2** were identical with those of natural **2**.

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5. In order to confirm and characterize spectroscopically the minor *syn* diastereomer of compound **1**, we also synthesized it in its racemic form. The NOE experiment for synthesized (±)-**17** (Fig. 4) clearly supported the proposed configuration, i.e., a clear NOE enhancement was observed between the two methine protons at $\delta$ 4.34 (H-5, H-5′) and 2.67 (H-3, H-3′). (±) −**17**: amorphous solid; IR (ATR) $\nu_{max}$ 2935, 1761 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 4.34 (2H, dddd, *J*=10.0, 7.5, 5.0, 5.0 Hz, H-5, H-5′), 2.67 (2H, m, H-3, H-3′), 2.61 (4H, dd, *J*=7.0, 7.0 Hz, $H_2$-9, $H_2$-9′), 2.49 (2H, ddd, *J*=12.4, 8.4, 5.6 Hz, H-4b, H-4′b), 1.74 (2H, m, H-6, H-6′), 1.65 (2H, m, H-6, H-6′), 1.47 (10H, m, H-4, H-4′, $H_2$-7, $H_2$-7′, $H_2$-8, $H_2$-8′), 1.27 (6H, d, *J*=7.0 Hz, $H_3$-11, $H_3$-11′); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 179.5 (C, C-2, C-2′), 78.5 (CH, C-5, C-5′), 49.8 ($CH_2$, C-9, C-9′), 37.3 ($CH_2$, C-4, C-4′), 35.9 ($CH_2$, C-3, C-3′), 35.4 (CH, C-6, C-6′), 29.9 ($CH_2$, C-8, C-8′), 23.2 ($CH_2$, C-7, C-7′), 15.1 ($CH_3$, C-11, C-11′); HR-FABMS: calcd for $C_{18}H_{32}NO_4$ $[M+H]^+$: 326.2331, found 326.2341.
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10. For reference, the chiral HPLC conditions (Daicel Chiralcel AD-H; hexane/EtOH 95/5; flow rate=0.5 mL/min; $\lambda$=254 nm; $t_{major}$=9.9 min., $t_{minor}$=12.6 min) were established using a racemic sample that was prepared in the same way using DL-proline as catalyst.
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12. In the $^1H$ NMR spectrum, two sets of peaks having a 7:1 integration for the H-5 proton were observed ($\delta$ 4.51, *anti* and 4.34, *syn*). The major diastereomer was assigned to be *anti* because a clear NOE peak enhancement was observed between the H-5 methine ($\delta$ 4.51) and the H-11 doublet methyl ($\delta$ 1.28).
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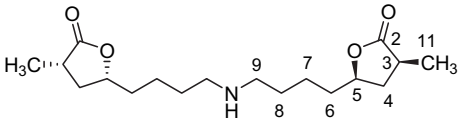


**Figure 4.** Structure of (±)-**17**, the minor *syn* diastereomer of **1**.