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.

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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.
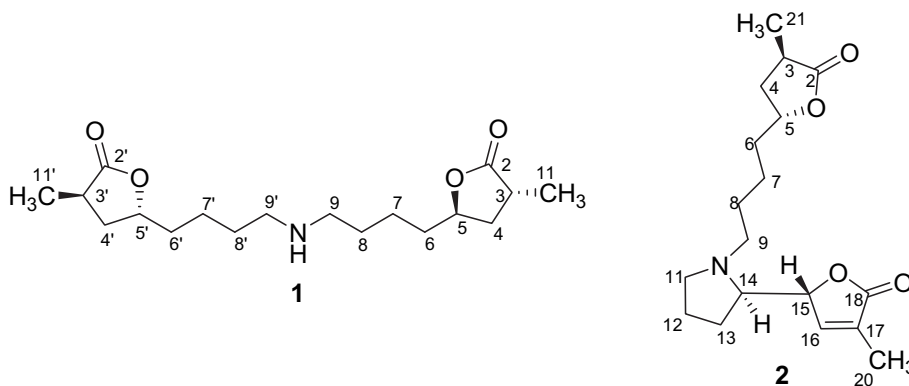

Figure 1. Structures of new alkaloids from *P. dubius*.

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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 {[α]$_D^{20}$ +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 (δ 180.1), an oxygenated methine (δ 78.3), a methylene associated to a nitrogen atom (δ 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 ^{1}H NMR spectrum showed a total count of 15 hydrogen atoms in the molecule. Supported by ^{1}H–^{1}H 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 (δ 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.
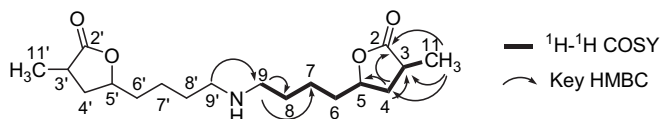
Examination of the structure of **1** revealed the presence of two stereocenters — one at C-3 and the other at C-5. Detailed analysis of both ^{1}H and ^{13}C NMR spectra showed the occurrence of an inseparable 5:1 diastereomeric mixture. In particular, additional minor signals at δ 4.34 and 2.49 (^{1}H) and δ 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 δ 4.51 (H-5, H-5′) led to a clear peak enhancement of the methyl protons at δ 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$_2$CO$_3$ and PhSH. The product was carefully purified using silica gel chromatography to afford diastereomerically pure **1** {[α]$_D^{23}$ +61.2 (*c* 0.05, CHCl$_3$)}. The spectroscopic data (IR, ^{1}H 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.

Figure 2. COSY and selected HMBC correlations of **1**.
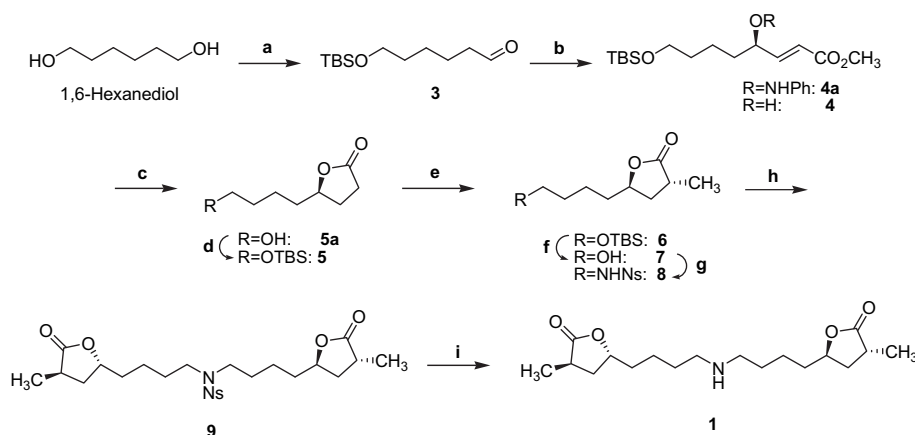
Scheme 1. Synthetic route for dubiusamine-A. Reagents and conditions: (a) (i) TBSCl, NaH, THF, rt, 3 h; (ii) SO$_3$-pyridine complex, DMSO, Et$_3$N, CH$_2$Cl$_2$, 0 °C to rt, 3 h, 80% in two steps; (b) (i) L-Proline, Nitrosobenzene, CH$_3$CN, −20 °C, 20 h; (ii) Methyl diethyl phosphonoacetate, NaH, THF, 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$_2$Cl$_2$, 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$_2$CO$_3$, PhSH, DMF/CH$_3$CN, rt, 3 h, 96%.

Dubiusamine-B (**2**) was obtained as an optically active, amorphous solid {$[\alpha]_D^{18}$ +13.6 (c 0.025, CHCl₃)}. 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, ¹³C (Table 1) and DEPT NMR spectra indicated two lactone carbonyls, five methines (two oxygen-bearing, one nitrogen-bearing and one sp²), eight methylenes (two nitrogen-bearing), two methyls, and one olefinic quaternary carbon. The observed ¹H and ¹³C NMR signals at {δ_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); δ_C 174.4 (C-18), 146.9 (C-16), 131.3 (C-17), 83.7 (C-15), 10.8 (C-20)} and at {δ_H 2.80 (ddd, J=8.0, 5.5, 5.0, H-14); δ_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 ¹³C 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 ¹³C NMR data for **2** in CDCl₃

| Position	δ_H (500 MHz)	δ_C (125 MHz)
2		180.0
3	2.70, m	34.0
4	2.03, ddd (10.0, 7.2, 6.6)	35.5[a]
	2.12, ddd (10.5, 7.0, 4.0)	
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)	55.6
	2.40, ddd (11.0, 7.5, 6.0)	
11	3.12, dd (7.5, 6.5)	54.3
	2.20, m	
12	1.70–1.80, overlapped	25.9
	1.46–1.63, overlapped	
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.
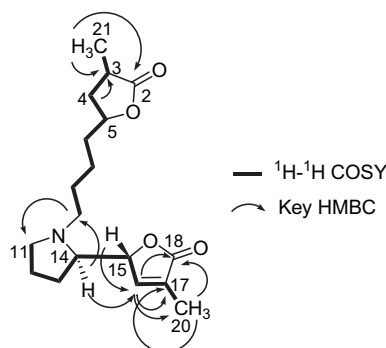
Figure 3. COSY and selected HMBC correlations of **2**.

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, 1S, erythro) configuration for the less polar adduct and (2′R, 1R, threo) configuration for the more polar adduct. Removal of the Cbz group in the more polar adduct using TMSI in CH₃CN afforded amino alcohol **13**. Condensation of **13** with the γ-butyrolactone iodide **10** having a 3R, 5R configuration in the presence of Ag₂CO₃ in CH₃CN led to the formation of intermediate adduct **14**. Esterification of the hydroxyl group in **14** with methacrylic anhydride in the presence of Et₃N and DMAP in CH₂Cl₂ 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, ¹³C NMR) including the high-resolution mass spectrometric and optical rotation {$[\alpha]_D^{17}$ +22 (c 0.30, CHCl₃)} data of the synthetic material, were in excellent agreement with those of the natural product. Thus, the structure including the absolute configuration (3R, 5R, 14R, 15R) 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 Al₂O₃ 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 Na₂CO₃ (pH 9–10) and exhaustively extracted with 5%
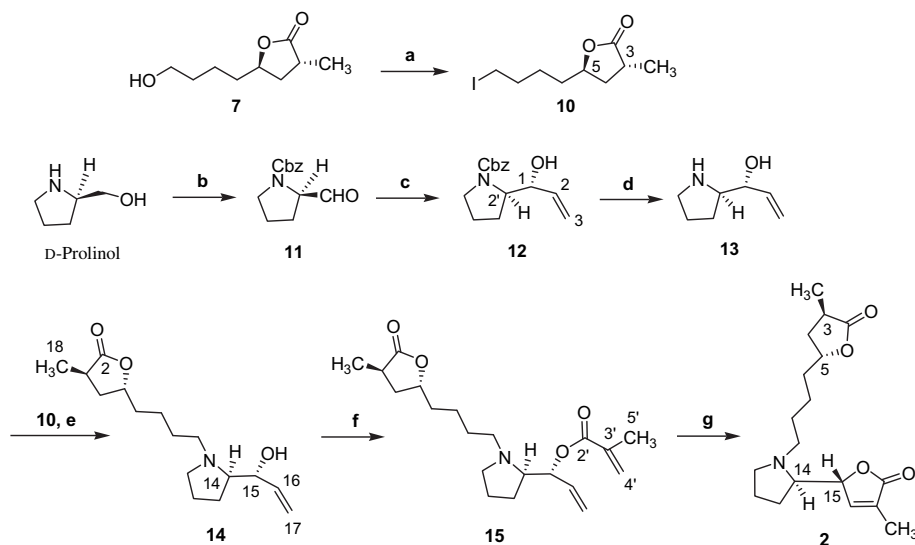
Scheme 2. Synthetic route for dubiusamine-B. Reagents and conditions: (a) I$_2$, PPh$_3$, Imidazole, CH$_2$Cl$_2$, rt, 3.5 h, 96%; (b) CbzCl, K$_2$CO$_3$, CH$_3$CN, −20 °C, 4 h; then SO$_3$–pyridine complex, DMSO, Et$_3$N, CH$_2$Cl$_2$, 0 °C, 3.5 h, 92% in two steps; (c)Vinyl magnesium bromide, THF, 0 °C, 3 h, 73% (dr 2:1); (d) TMSI, CH$_3$CN, −15 °C to 0 °C, 30 min 53%; (e) **10**, Ag$_2$CO$_3$, CH$_3$CN, rt, 45 h, 65%; (f) Methacrylic anhydride, Et$_3$N, DMAP, CH$_2$Cl$_2$, rt, 4 h, 71%; (g) *p*-TsOH, Grubbs second-generation catalyst, CH$_2$Cl$_2$, reflux, 19 h, 61%.
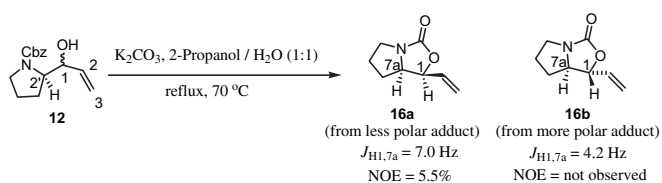
Scheme 3. Determination of absolute configuration.

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; [α]$_D^{20}$ +29.5 (c 0.07, CHCl$_3$); IR (ATR) ν_{max} 2936, 1759, 1160 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 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) δ 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; [α]$_D^{18}$ +13.6 (c 0.025, CHCl$_3$); UV λ$_{max}$ 209, 243, 273 nm; ^{1}H 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$_3$CN (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$_4$Cl, 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$_4$Cl 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) λ_{max} 206 nm; IR (ATR) ν_{max} 3437, 2929, 2857, 1725, 1659, 1471, 1253 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_4$Si, [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$_2$Cl$_2$ (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$_4$Cl 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) ν_{max} 2922, 2858, 1750, 1213 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_3$Si [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) ν_{max} 3419, 2934, 2866, 1752, 1455, 1185 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_9$H$_{16}$O$_3$Na [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-nitro-benzenesulfonamide (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) λ_{max} 204, 221 (sh) nm; IR (ATR) ν_{max} 2935, 2866, 1755, 1537, 1338, 1161 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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, NH), 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$_3$) δ 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$_2$O$_6$NaS [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) λ_{max} 206 nm; IR (ATR) ν_{max} 2937, 1759, 1542, 1455, 1159 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_2$O$_8$NaS [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$_3$CN (80 μL) were added K$_2$CO$_3$ (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$_2$Cl$_2$ (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$_2$S$_2$O$_3$ (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) ν_{max} 1755 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_9$H$_{15}$O$_2$INa [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$_4$Cl 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) δ 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, OH), 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) δ 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) ν_{max} 3422, 2975, 1671, 1497, 1412 cm^{-1}; ^{1}H NMR (DMSO-d_6, VT 100, 400 MHz) δ 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, OH), 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) δ 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$_3$Na [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$_3$CN (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$_2$O$_3$ 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) ν_{max} 3316,

2966, 2875, 1631, 1536 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 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) δ 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$_7$H$_{14}$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$_3$CN (1.0 mL) was added Ag$_2$CO$_3$ (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) δ 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) δ 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$_2$Cl$_2$ (0.41 mL) at 0 °C were added Et$_3$N (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$_4$Cl 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) δ 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) δ 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$_2$Cl$_2$ (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$_2$Cl$_2$ 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**.

Acknowledgements

References and notes

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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 *δ* 4.34 (H-5, H-5′) and 2.67 (H-3, H-3′). (±)−**17**: amorphous solid; IR (ATR) *ν*$_{max}$ 2935, 1761 cm^{-1}; ^1H NMR (CDCl₃, 500 MHz) *δ* 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, H₂-9, H₂-9′), 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′, H₂-7, H₂-7′, H₂-8, H₂-8′), 1.27 (6H, d, *J*=7.0 Hz, H₃-11, H₃-11′); ^{13}C NMR (CDCl₃, 125 MHz)

δ 179.5 (C, C-2, C-2′), 78.5 (CH, C-5, C-5′), 49.8 (CH₂, C-9, C-9′), 37.3 (CH₂, C-4, C-4′), 35.9 (CH₂, C-3, C-3′), 35.4 (CH, C-6, C-6′), 29.9 (CH₂, C-8, C-8′), 23.2 (CH₂, C-7, C-7′), 15.1 (CH₃, C-11, C-11′); HR-FABMS: calcd for $C_{18}H_{32}NO_4$ $[M+H]^+$: 326.2331, found 326.2341.
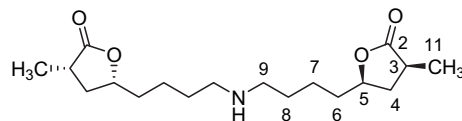

Figure 4. Structure of (±)-**17**, the minor *syn* diastereomer of **1**.

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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; λ=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 (*δ* 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 (*δ* 4.51) and the H-11 doublet methyl (*δ* 1.28).
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