Total synthesis of dubiusamine C, a plausible minor alkaloid in *Pandanus dubius*

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Dubiusamine C is a minor alkaloid isolated as a diastereomeric mixture with dubiusamine A. The structure of dubiusamine C was deduced by ^{1}H-NMR analysis and then identified by its racemic total synthesis which employed the Grignard, ring-closing metathesis, stereoselective reduction and Mitsunobu reactions as the key steps, in nine linear steps and an over-all yield of 20%.

KEYWORDS

Alkaloid, Pandanaceae, Pandanus, Pyrrolidine, Structure Elucidation, Total Synthesis

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INTRODUCTION

The genus *Pandanus* (Pandanaceae) comprises about 700 species that are distributed in tropical and subtropical regions. Previous phytochemical studies on the Pandanus species have elaborated the presence of alkaloids, terpenes, lignans, and essential oils (Nonato et al. 2008). In our continuing search for structurally-unique alkaloids from the genus Pandanus (Tan et al. 2010a, Tan et al. 2010b), we have recently investigated the leaves of *Pandanus dubius* (Tan et al. 2010c). As a result, two new alkaloids, dubiusamines A and B, and seven known alkaloids, were identified by spectroscopic methods and confirmed by total synthesis.

Dubiusamine A (**1**) is a symmetrical secondary amine having a *trans* stereochemical relationship of its H-3 and H-5 methines. It was identified by 1D and 2D NMR, NOE and HR-MS analyses. Its structure, including the absolute configuration, was unambiguously determined by its asymmetric total synthesis (Tan et al. 2010C). The isolated natural product, however, proved to be a mixture which is comprised of 4:1 diastereomers. Its minor diastereomer, named dubiusamine C (**2**), was hypothesized to contain a *syn* relationship between the H-3 and H-5 methine protons. In this paper, the racemic total synthesis of minor alkaloid **2** was carried out to efficiently characterize its structure by spectroscopic techniques. Our synthetic strategy

employed a Grignard, ring-closing metathesis, stereoselective reduction and Mitsunobu reactions as the key steps.

MATERIALS AND METHODS

General experimental procedures

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. *m*-Nitrobenzyl alcohol (NBA) was used as the matrix. HRESIMS were recorded on a Thermo Fisher Scientific Exactive spectrometer. NMR spectra were recorded on JEOL JNM A-500 or JEOL JNM ECP400 spectrometers. The chemical shifts are given in δ (ppm) and coupling constants, in Hz. Kieselgel 60 [Merck, 70-230 mesh (for open column chromatography)] or Silica gel 60N [Kanto Chemical, 40-50 µm (for flash column chromatography)] were used for column chromatography. Medium-pressure liquid chromatography was carried out on a silica gel prepacked column CPS-HS-221-05 (Kusano Kagakukikai).

Preparation and characterization of new compounds

Preparation of benzyloxy methacrylate (6)

To a stirred solution of benzyloxy heptenol (Iyengar et al. 2005), **5** (36.1 mg, 0.164 mmol) in CH_2Cl_2 (350 µL) at 0 ºC was added Et_3N (68 µL, 0.492 mmol, 3 eq) dropwise and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP) (4 mg, 0.033 mmol). Methacryloyl chloride (35.2 µL, 0.361 mmol, 2.2 eq) was added dropwise and the resulting mixture was allowed to stir at room temperature for 3.5 h. The reaction mixture was quenched with saturated NH_4Cl and 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 flash column chromatography (5% EtOAc in hexane) afforded compound **6** as colorless oil in 66% yield (31.0 mg); IR (ATR) ν_{max} 1715, 1633, 1501 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 7.36-7.28 (5H, m, Phenyl group), 6.11 (1H, d, *J*=1.6 Hz, H-3'a), 5.81 (1H, ddd, *J*=6.4, 10.4, 16.4 Hz, H-2), 5.55 (1H, d, *J*=1.6 Hz, H-3'b), 5.30 (1H, q, *J*=6.4 Hz, H-3), 5.25 (1H, br d, *J*=16.4 Hz, H-1a), 5.17 (1H, br d, *J*=10.4 Hz, H-1b), 4.50 (2H, s, -OC*H*$_2$-), 3.47 (2H, t, *J*=6.0 Hz, H-7), 1.93 (3H, s, H_3-4'), 1.75-1.39 (6H, m, H_2-4, H_2-5, H_2-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 166.6 (C, C-1'), 138.5 (C), 128.3 (CH), 127.6 (CH), 127.5 (CH) [Phenyl group], 136.6 (CH, C-2), 136.5 (C, C-2'), 125.2 (CH_2, C-3'), 116.5 (CH_2, C-1), 72.8 (-O*C*H$_2$-), 70.0 (CH_2, C-7), 74.8 (CH, C-3), 34.0 (CH_2, C-4), 29.4 (CH_2, C-6), 21.7 (CH_2, C-5), 18.3 (CH_3, C-4'); FAB-MS (NBA): *m/z* 289 [M+H].

Preparation of benzyloxy butyrolactone (7)

To a stirred solution of **6** (155 mg, 0.538 mmol) in CH_2Cl_2 was added Grubbs II catalyst (33 mg, 0.0534 mmol, 0.1 eq). The mixture was refluxed for 7 h at 60 ºC. After cooling, the solvent was concentrated under reduced pressure. Purification by silica gel flash column chromatography (30% EtOAc in hexane) afforded compound **7** as yellow oil in 93 % yield (130 mg); UV

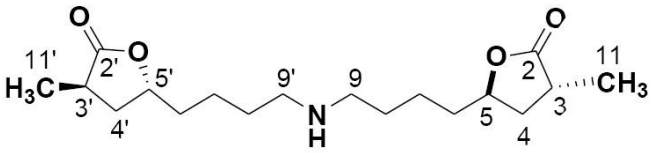

Figure 1. Dubiusamine A (**1**)

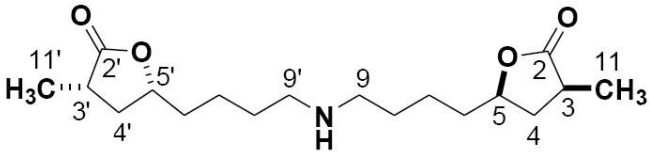

Figure 2. Proposed structure of Dubiusamine C (**2**)

(MeOH) λ_{max} 276, 216 nm; IR (ATR) ν_{max} 1765, 1551 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 7.37-7.28 (5H, m, Phenyl group), 7.02 (1H, br s, H-4), 4.87 (1H, m, H-5), 4.49 (2H, s, -OC*H*$_2$-), 3.47 (2H, t, *J*=6.2 Hz, H-9), 1.91 (3H, d, *J*=1.2 Hz, H_3-10), 1.73-1.52 (6H, m, H_2-6, H_2-7, H_2-8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 174.2 (C, C-2), 148.6 (C-4), 138.4 (C-3), 129.9, 128.3, 127.6, 127.5 (Phenyl group), 81.0 (C-5), 72.9 (-O*C*H$_2$-), 69.8 (C-9), 33.2 (C-8, 29.4 (C-6), 21.9 (C-7), 10.6 (C-10) FAB-MS (NBA): *m/z* 261[M+H]$^+$.

Preparation of butyrolactonol (8)

To a stirred solution of **7** (100 mg, 0.384 mmol) in MeOH (8 mL) was added 10% Pd/C (50 mg). The reaction mixture was stirred under hydrogen atmosphere (balloon) for 14 h and filtered using a pad of Celite (EtOAc). The filtrate was concentrated under reduced pressure to give the crude residue (69.5 mg). The crude residue was purified by silica gel flash column chromatography (EtOAc) to afford the compound **8** as colorless oil in quantifiable yield (65.8 mg); IR (ATR) ν_{max} 3560, 1755, 1451 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 4.35 (1H, m, H-5), 3.67 (2H, t, *J*=6.2 Hz, H-9), 2.67 (1H, m, H-3), 2.49 (1H, ddd, *J*=12.4, 8.4, 5.2 Hz, H-4a), 1.81 (1H, m, H-4b), 1.70-1.48 (6H, m, H_2-6, H_2-7, H_2-8), 1.27 (3H, d, *J*=6.8 Hz, H_3-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 179.5 (C, C-2), 78.5 (CH, C-5), 62.6 (CH_2, C-9), 37.3 (CH_2, C-4), 35.9 (CH_2, C-6), 35.2 (CH, C-3), 32.3 (CH_2, C-8), 21.7 (CH_2, C-7), 15.1 (CH_3, C-11); FAB-MS (NBA): *m/z* 173 [M+H]$^+$.

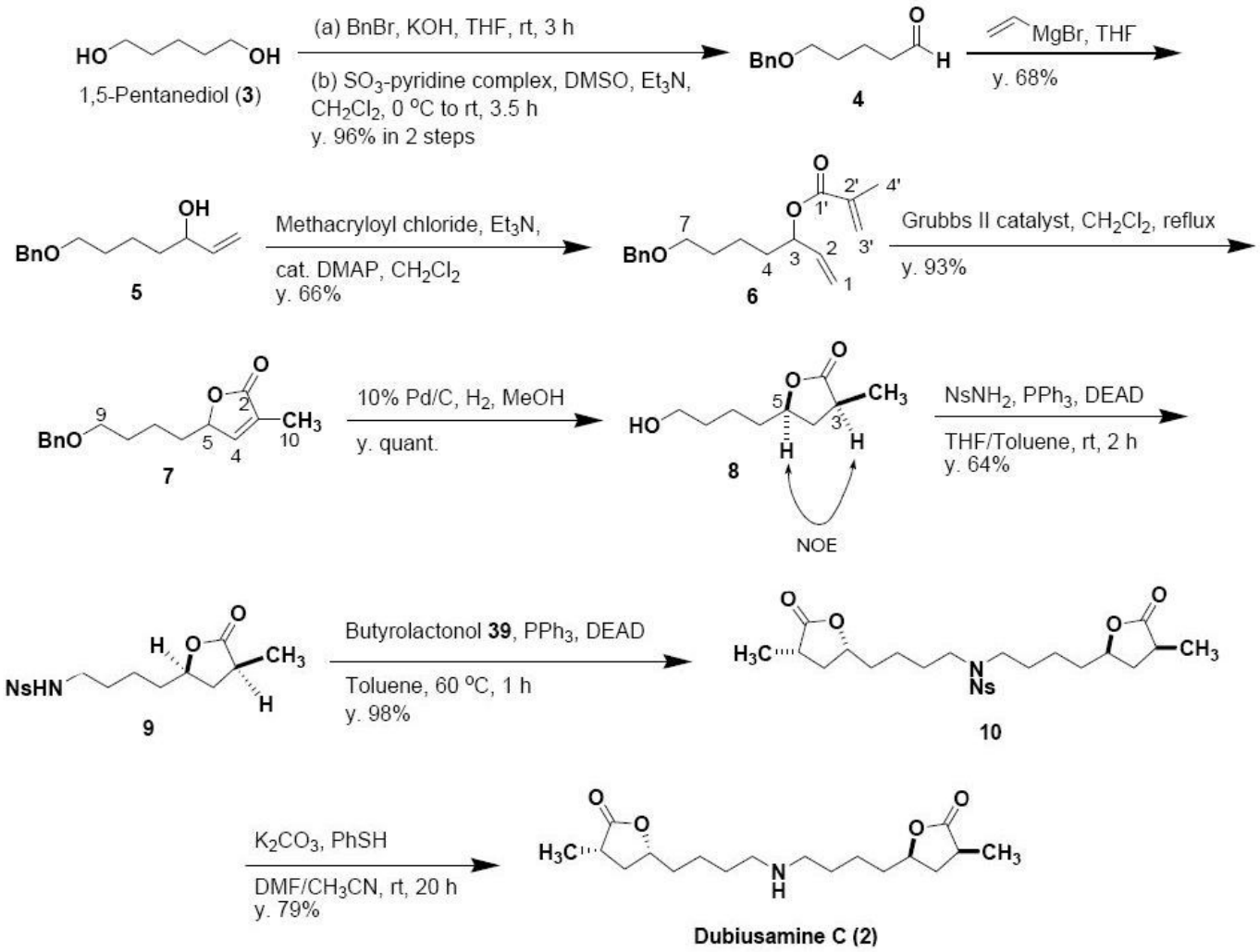

Figure 3: Synthetic route for dubiusamine C (**2**)

Preparation of *syn*-amine (9)

To a stirred solution of **8** (86 mg, 0.500 mmol) in THF (7.0 mL) and toluene (3.0 mL) were added 2-nitrobenzenesulfonamide ($NsNH_2$) (253 mg, 1.25 mmol, 2.5 eq) and triphenylphosphine (170.5 mg, 0.650 mmol, 1.3 eq). The solution was allowed to cool in an ice bath and diethyl azodicarboxylate (DEAD) (40% in toluene, 283 µL, 0.650 mmol, 1.3 eq) was added dropwise. The reaction mixture was stirred at room temperature for 2 h. The mixture was filtered using a Celite pad, and the filtrate was concentrated under reduced pressure. Purification by silica gel flash column chromatography (hexane/EtOAc/$CHCl_3$ 1:0.5:0.5) afforded the compound **9** as a light-yellow oil in 64% yield (112 mg); UV (MeOH) λ_{max} 205 nm; IR (ATR) ν_{max} 1755, 1537 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.14 (1H, m, Nosyl group), 7.88 (1H, m, Nosyl group), 7.76 (2H, m, Nosyl group), 5.27 (1H, t, *J*=5.8 Hz, -N*H*), 4.29 (1H, m, H-5), 3.11 (2H, qd, *J*=6.4, 1.8 Hz, H-9), 2.65 (1H, m, H-3), 2.47 (1H, ddd, *J*=12.4, 8.8, 5.6 Hz, H-4), 1.69-1.41 (7H, m, H-4, H_2-6, H_2-7, H_2-8), 1.26 (3H, d, *J*=7.2 Hz, H_3-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 179.3 (C, C-2), 148.1 (C), 133.6 (CH), 133.6 (CH) 132.8 (C), 131.0 (CH), 125.4 (CH) [Nosyl group], 78.2 (CH, C-5), 43.5 (CH_2, C-9), 37.2 (CH_2, C-4), 35.8 (CH, C-3), 34.9 (CH_2, C-6), 29.3 (CH_2, C-8), 22.4 (CH_2, C-7), 15.0 (CH_3, C-10); FAB-MS (NBA): *m/z* 357 [M+H]; HRESIMS: calcd for $C_{15}H_{20}N_2O_6NaS$ $[M+Na]^+$: 379.0934, found: 379.0929.

Preparation of symmetrical *syn*-amide (10)

To a stirred mixture of **9** (20 mg, 0.0561 mmol), **8** (14.5 mg, 0.0842 mmol, 1.5 eq) and triphenylphosphine (19.1 mg, 0.0729 mmol, 1.3 eq) in toluene (353 µL) was added DEAD (40% in toluene, 31.8 µL, 1.3 eq, 0.0729 mmol) dropwise at 0 °C. 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

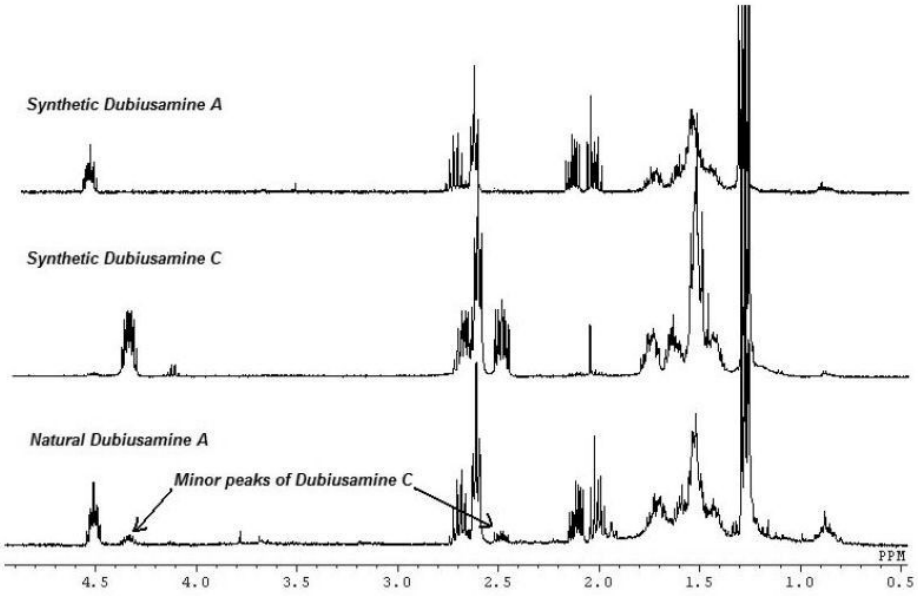

Figure 4. 1H NMR Spectra (500 MHz, CDCl3) of Compounds 1 (Tan et al. 2010c) and 2.

Table 1. 1H (500 MHz) and 13C (125 MHz) NMR data for **2**a and **1**b in CDCl3

	2 (δ_H)	**1** (δ_H)	**2** (δ_C)	**1** (δ_H)
2, 2'			179.5	180.1
3, 3'	2.67, 2H, m	2.69 (2H, m)	35.9	34.0
4, 4'	2.49, 2H, ddd (12.4, 8.4, 5.6)	2.12, 2H, ddd (10.4, 6.6, 4.0)	37.3	35.5
	1.47, 2H, overlapped	2.00, 2H, ddd (10.4, 6.0, 6.0)		
5, 5'	4.34, 2H, dddd (10.0, 7.5, 5.0, 5.0)	4.51, dddd (13.0, 8.0, 8.0, 5.0)	78.5	78.3
6, 6'	1.74, 2H, m	1.70, 2H, m	35.4	35.4
	1.65, 2H, m	1.60 – 1.46, 2H, overlapped		
7, 7'	1.47, 4H, overlapped	1.60 – 1.46, 4H, overlapped	23.2	23.3
8, 8'	1.47, 4H, overlapped	1.60 – 1.46, 4H, overlapped	29.9	29.7
9, 9'	2.61, 4H, dd (7.0, 7.0)	2.61, 4H, dd (6.8, 6.8)	49.8	49.7
11, 11'	1.27, 6H, d (7.0)	1.28, 6H, d (7.0)	15.1	15.9

[a] Determined by 2D NMR (COSY, HMQC, HMBC).
[b] Tan et al. 2010c.

concentrated under reduced pressure. The crude material was purified by silica gel flash column chromatography (50% EtOAc in hexane – 90% EtOAc in hexane) to obtain compound **10** as a light-yellow amorphous solid in 98% yield (28 mg); UV (MeOH) λ_{max} 206 nm; IR (ATR) ν_{max} 1759, 1542, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.00 (1H, m, Nosyl group), 7.71 (2H, m, Nosyl group), 7.62 (1H, m, Nosyl group), 4.30 (2H, H-5, H-5'), 3.29 (4H, m, H_2-9, H_2-9'), 2.67 (2H, m, H-3, H-3'), 2.48 (2H, ddd, J=12.8, 8.8 5.6 Hz, H-4, H-4'), 1.70-1.40 (14H, m, H-4, H-4', H_2-6, H_2-6', H_2-7, H_2-7', H_2-8, H_2-8'), 1.26 (6H, d, J=6.8 Hz, H_3-11, H_3-11'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 179.4 (C, C-2, C-2'), 147.9 (C), 133.5 (CH), 133.3 (C), 131.6 (CH), 130.6 (CH), 124.1 (CH) [Nosyl group], 78.2 (CH, C-5, C-5'), 47.1 (CH2, C-9, C-9'), 37.2 (CH_2, C-4, C-4'), 35.8 (CH, C-3, C-3'), 34.9 (CH_2, C-6, C-6'), 27.9 (CH_2, C-8, C-8'), 22.5 (CH_2, C-7, C-7'), 15.0 (CH_3, C-11, C-11'); FAB-MS (NBA): *m/z* 511 $[M+H]^+$; HRESIMS: calcd for $C_{24}H_{34}N_2O_8NaS$ $[M+Na]^+$: 533.1928, found: 533.1920.

Synthesis of the minor alkaloid dubiusamine C (2)

To a stirred solution of **10** (14 mg, 0.0274 mmol) and K_2CO_3 (7.6 mg, 0.0548 mmol, 2 eq) in DMF (30 µL) and CH_3CN (40 µL) was added thiophenol (3.7 µL, 0.036 mmol, 1.3 eq) and the mixture was stirred at room temperature for 20 h. The reaction mixture was filtered using a Celite pad and the filtrate was concentrated under reduced pressure. Title compound **2** was afforded after purification using silica gel open column chromatography (10% MeOH* /$CHCl_3$; M* = 10% NH_3 in MeOH) as an amorphous solid in 79 % yield (7 mg); IR (ATR) ν_{max} 1755 cm^{-1}; ^1H and ^{13}C data, see Table 1; HRFABMS: calcd for $C_{18}H_{32}NO_4$ $[M+H]^+$: 326.2331, found: 326.2341.

RESULTS and DISCUSSION

The total synthesis of dubiusamine C (**1**) (Figure 3) was initiated by the monoprotection of 1,5-pentanediol (**3**) with benzyl bromide (Kiddie et al. 1995). The remaining hydroxy group was oxidized to an aldehyde using the SO_3-pyridine procedure to obtain the benzyloxy pentanal (**4**) (Chen et al. 2005) as colorless oil in 96% yield over two steps. The installation of the vinyl group in compound **4** using vinyl magnesium bromide in THF yielded the benzyloxy heptenol (**5**) in 68% yield (Iyengar et al. 2005). Esterification (Cho et al. 2005) of the hydroxyl group in compound **5** was attempted using methacryloyl chloride, Et_3N, and catalytic amount of DMAP in CH_2Cl_2 to give the benzyloxy methacrylate (**6**) as colorless oil in 66% yield. Ring-closing metathesis (Bogliotti et al. 2006) of **6** to form the α-methyl-α,β-unsaturated-γ-lactone unit in benzyloxy butyrolactone (**7**) was easily accomplished by reflux using Grubbs second generation

catalyst in CH_2Cl_2 in 93% yield. Hydrogenation using 10% Pd/C and hydrogen gas in MeOH of the benzyloxy butyrolactone (**7**) resulted in the removal of the benzyl protecting group and stereoselective reduction of the olefinic group to obtain butyrolactonol (**8**) in quantitative yield. The absence of the NMR signals of the benzyl and the olefinic groups supported the structure of butyrolactonol. At this stage, the *syn* relationship of the H-3 (δ_H 2.67, 1H, m) and H-5 (δ_H 4.35, 1H, m) methine protons was elucidated by their strong NOE correlation (3.2%). Then the Mitsunobu reaction to obtain the *syn*-amine (**9**) proceeded by treating the butyrolactonol (**8**) with 2-nitrobenzenesulfonamide, PPh_3 and DEAD in THF/toluene. Construction of the symmetrical compound *syn*-amide (**10**) went on efficiently using a reaction mixture composed of the *syn*-amine (**9**), butyrolactonol (**8**), PPh_3 and DEAD in toluene, as attested by the FAB-MS data at *m/z* 511 $[M+H]^+$. To conclude the total synthesis, deprotection of the nosyl group in *syn*-amide (**10**) using PhSH and K_2CO_3 in DMF and CH_3CN gave dubiusamine C (**2**) in 79% yield. The synthesis was accomplished in a total of nine linear steps and an over-all yield of 20%.

Comparison of the NMR spectrum (Figure 4) for natural dubiusamine-A (**1**) and the synthesized dubiusamine C (**2**) confirmed that the minor peaks [δ 4.34 and 2.49 (^{1}H) and δ 37.3, 35.9, and 15.1 (^{13}C)] observed in the NMR chart of natural **1** corresponded to those of **2**. Moreover, the NOE correlation (3.6%) of the methine protons of 2 at H-3 (δ_H 2.67) and H-5 (δ_H 4.34) affirmed their *syn* relationship. Therefore, the relative structure of the minor diastereomer of **1** having a *syn* stereochemistry protons was confirmed as that of dubiusamine C (**2**).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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