New Indole Alkaloids from the Bark of *Alstonia scholaris*

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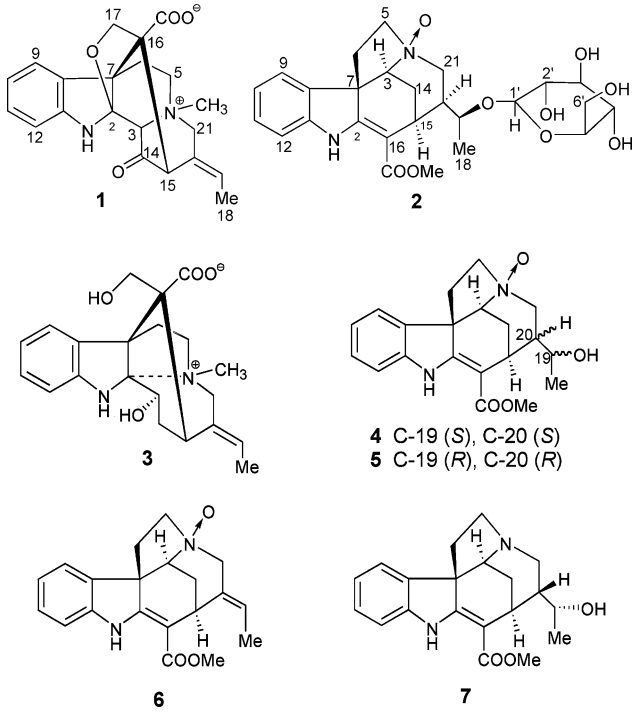
A new indole alkaloid, akuammiginone (**1**), and a new glycosidic indole alkaloid, echitamidine-*N*-oxide 19-*O*-*β*-D-glucopyranoside (**2**), together with the five known alkaloids, echitaminic acid (**3**), echitamidine *N*-oxide (**4**), N^b-demethylalstogustine *N*-oxide (**5**), akuammicine *N*-oxide (**6**), and N^b-demethylalstogustine (**7**), were isolated from the trunk bark of *Alstonia scholaris* collected in Timor, Indonesia. The structures of all compounds were elucidated by spectroscopic methods. This is the first report of compounds **3**−**5** and **7** in *A. scholaris*. Some NMR assignments of the known compounds were revised.

Alstonia scholaris R. Br. (Apocynaceae) is widely distributed in South and Southeast Asia. The bark of this tree is used in traditional medicines throughout the region to treat dysentery and malaria.[1] The leaves of *A. scholaris* collected in India, Pakistan, Thailand, the Philippines, Malaysia, and Indonesia have been investigated and show diverse alkaloidal patterns.[2−15] In general, the continental trees (India, Pakistan, Thailand) contain picrinine-type alkaloids, while those from Indonesia and the Philippines contain alkaloids based on the angustilobine skeleton.[13] Trees from within Indonesia also show variation in the leaf alkaloid chemistry; leaf extracts from Java (Cianjur) contain scholaricine, while leaf extracts from Lombok contain tubotaiwine.[16]

Previous studies from Indian, Thailand, and Filipino specimens of *A. scholaris* showed that the major alkaloid obtained from the bark is echitamine (isolated as a chloride).[14,17−19] Echitamine chloride had been shown to have antitumor effects on fibrosarcoma in rats[20] and cytotoxic effects on Ehrlich ascites carcinoma cell cultures.[21] Bioactivity studies on bark extracts have shown that they have immunostimulating[22] and hepatoprotective[23] effects in mice and antifertility effects in male rats.[24] However, the active components responsible for these bioactivities have not yet been isolated.

The alkaloidal content of the bark of *A. scholaris* from Indonesia has not been investigated previously. In this paper we report the isolation and structural identification of indole alkaloids from the bark of *A. scholaris* collected from Timor, Indonesia. Two new alkaloids, akuammiginone (**1**) and echitamidine-*N*-oxide 19-*O*-*β*-D-glucopyranoside (**2**), were isolated, together with five known alkaloids, namely, echitaminic acid (**3**), echitamidine *N*-oxide (**4**), N^b-demethylalstogustine *N*-oxide (**5**), akuammicine *N*-oxide (**6**), and N^b-demethylalstogustine (**7**). The structures of the new alkaloids were elucidated on the basis of analysis of spectral data. To the best of our knowledge, this is the first report of the occurrence of **3**−**5** and **7** in *A. scholaris*.

Akuammiginone (**1**) was isolated as a light yellow amorphous solid with a molecular formula of $C_{21}H_{22}N_2O_4$ (HRESIMS). ^{13}C NMR and HSQC data showed the presence of two methyls, four methylenes, seven methines, and eight quaternary carbon atoms. Characteristic ^1H and ^{13}C NMR signals [*δ* 7.39 (1H, dd, *J* = 7.6, 1.0 Hz, H-9), 7.10 (1H, dt, *J* = 7.6, 1.0 Hz, H-11), 6.83 (1H, dd, *J* = 7.6, 1.0 Hz, H-12), 6.79 (1H, dt, *J* = 7.6, 1.0 Hz, H-10); 148.6 (C-13), 134.8 (C-8), 129.7 (C-11), 124.7 (C-9), 121.9 (C-10), 113.0 (C-12)] indicated the presence of an indole moiety. Downfield signals in the ^{13}C NMR spectrum at *δ* 189.9 and 174.3 suggested the presence of carbonyl and carboxyl functionalities, respectively. In the ^1H NMR spectrum, a doublet at *δ* 1.83 (3H, *J* = 7.0 Hz) was assigned as Me-18 and was scalar coupled to an olefinic proton at *δ* 5.79 (H-19), allowing the assignment of an ethylidene side chain. The downfield signals at C-3, C-5, and C-21 (*δ* 79.6, 63.3, and 69.1, respectively) suggested that they were adjacent to a quaternary nitrogen. A signal at *δ* 3.34 (s, 3H) and *δ* 55.4 was characteristic of an N^+-Me, and the methyl was connected to N-4 on the basis of HMBC cross-peaks to C-3, C-5, and C-21. Two methylene groups (*δ* 3.61, 3.26 and 4.13, 1.78) were assigned as H-5*α*/*β* and H-6*α*/*β* on the basis of DQF-COSY and HSQC data and were located between N-4 and C-7 on the basis of the HMBC data. A quaternary carbon at *δ* 132.6 (C-20) showed HMBC cross-peaks from H-15, H-18, and H-21*α*/*β* and allowed the assignment of the fragment N(4)−CH_2−C(CHMe)−CH−. The downfield shifts of the isolated methylene signals at *δ* 3.85 and 4.03 (H-17*α*/*β*) attached to a carbon at *δ* 81.9 suggested that



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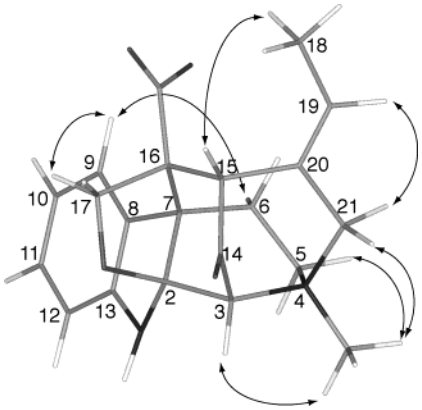

Figure 1. Selected NOE correlations for compound **1**.

this methylene is adjacent to both an oxygen atom and a quaternary carbon. In the HMBC spectrum, H17α/β showed correlations to C-2, C-7, C-14, C-15, C-16, and C-20, hence suggesting an akuammigine-type carbon skeleton. With all of the proton signals accounted for, the carboxyl group at δ 174.3 is in the form of COO^- and could be placed adjacent to C-16 on the basis of the HMBC cross-peak between COO^- and H-17β. The carbonyl group at δ 189.9 was consistent with the presence of a ketone functionality, which was confirmed by a chemical test with DNP (2,4-dinitrophenylhydrazine). This signal was assigned to C-14 on the basis of a HMBC cross-peak from H-3. Furthermore, a long-range ^4J coupling (7.4 Hz) was also observed between H-3 and H-15, and together with HSQC and HMBC data established the presence of the C-3, C-14, and C-15 fragment. The rather large ^4J coupling between H-3 and H-15 has precedent when a "*W*" arrangement of bonds is present in a rigid molecule[25] and is also probably enhanced in size by the carbonyl moiety at C-14.

The relative stereochemistry of **1** was determined from a 2D ROESY NMR experiment. The three-dimensional structure of **1** and some selected NOEs are shown in Figure 1. The molecule is likely to be very rigid due to the presence of six rings, which are fused together at C-2, C-3, N-4, C-7, C-15, and C-16. The non-indole part of the molecule forms a cage-like structure. ROESY cross-peaks were observed between H-9/H-6α, H-9/H-17α, H-3/N(4)-Me, H-5α/N(4)-Me, H-21β/N(4)-Me, and H-5α/H-21α and are consistent with 2*S*, 3*R*, 4*S*, 7*S*, 15*S*, and 16*R* relative stereochemistry. NOEs were observed between H-15/Me-18 and H-19/H-21α, suggesting the *E* configuration about the C-19−C-20 double bond.

Compound **2** was isolated as a light yellow amorphous solid with a molecular formula of $C_{26}H_{34}N_2O_9$ (HRESIMS). ^{13}C and DEPT NMR data showed the presence of one methyl, five methylenes, 14 methines, and six quaternary carbons. Characteristic ^1H and ^{13}C NMR signals [δ 7.50 (1H, dd, J = 7.4, 1.0 Hz, H-9), 7.21 (1H, dt, J = 7.6, 1.0 Hz, H-11), 6.96 (1H, dt, J = 7.4, 1.0 Hz, H-10), 6.70 (1H, dd, J = 7.6, 1.0 Hz, H-12); 145.5 (C-13), 133.9 (C-8), 130.2 (C-11), 122.4 (C-10), 121.8 (C-9), 111.6 (C-12)] indicated the presence of an indole moiety. A quaternary carbon at δ 168.2, showing an HMBC correlation to the carboxymethyl protons at δ 3.79 (3H, s), indicated the presence of a methyl ester function. The downfield ^{13}C NMR shift at δ 79.2 (C-3), 68.8 (C-5), and 66.2 (C-21) and the lack of an N(4)-methyl signal indicated that the quaternary nitrogen was present as an N(4)-oxide. The methylene signals of H-5α/β (δ 3.73, 3.84) and H-6α/β (δ 2.27, 2.42) are scalar coupled and were placed between C-7 and N-4 on the basis of the HMBC data. The molecular fragment C-3 to C-21 through C-14, C-15, and C-20 was constructed on the basis of the DQF-COSY and HSQC data. The DQF-COSY and HSQC data also established the presence of a branched chain carbon sequence from C-18 to C-20. A signal at δ 104.9 (C-16), showing HMBC cross-peaks to OMe, H-14a/b, H-15, and H-20, is a characteristic of a polarized double bond connected to C-2 (δ 164.9) in the echitamidine skeleton.[26] From the remaining proton signals, a sugar moiety was constructed and was connected to the aglycone through C-19 (δ 75.8). The large coupling constant between H-1′ and H-2′ (7.8 Hz) indicated that these protons are in a *trans*-diaxial relationship to each other. The sugar unit was identified as a glucose residue on the basis of the value of ^3J coupling between the various sugar protons. The ^1H and ^{13}C NMR shifts and the coupling constants of the sugar unit were comparable to the published values for β-D-glucose.[27]

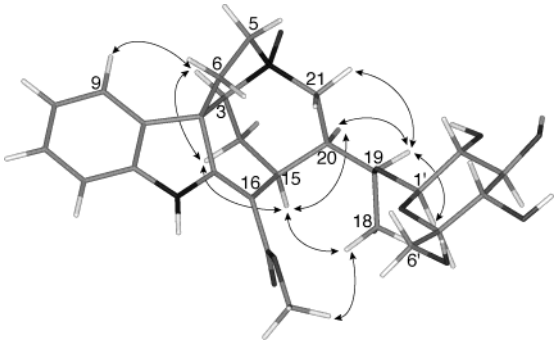

Figure 2. Selected NOE correlations for compound **2**.

The relative stereochemistry of **2** was determined from a 2D NOESY NMR experiment. The three-dimensional structure of **2** and some selected NOEs are shown in Figure 2. The proton at C-3 showed NOESY cross-peaks to H-9 and H-6α, suggesting that the relative stereochemistry at C-3 is *S*. NOESY cross-peaks were also observed between H-3/H-14a, H-3/H-14b, H-14a/H-15, and H-14b/H-15, indicating that C-15 is in the *S* configuration relative to C-3. A large coupling constant (J = 13.5 Hz) was observed between H-20 and H-21β, suggesting that they are in a *trans*-diaxial relationship to each other.[28] Moreover, H-20 also showed NOESY cross-peaks to H-15, H-18, H-19, and H-21α, suggesting that C-20 is in the *S* configuration. The relative stereochemistry at C-19 was determined as *S*, on the basis of observed NOESY cross-peaks between Me-18/OMe, Me-18/H-15, H-19/H-15, H-19/H-20, H-19/H-21α, and H-19/H-21β. Although there is the potential for rotation about bonds between some of these pairs of protons, free rotation is not likely because of the bulky surrounding groups. Even taking into account some rotations, the observed relative NOE intensities were consistent only with the *S* and not the *R* configuration. NOEs were observed between H-1′/H-18 and H-1′/H-19, suggesting that H-1′ is located at the same side of the molecule as H-19. As expected for glucose, NOEs were also observed between H-1′/H-3′, H-1′/H-5′, H-3′/H-5′, and H-2′/H-4′. On the basis of the NOESY data and the negative value of $[\alpha]_D$ (−148.4°, *c* 0.35, MeOH), the aglycone has the same configuration as echitamidine (C-19 *S*, C-20 *S*).[29] Hence the new alkaloid was named echitamidine-*N*-oxide 19-*O*-β-D-glucopyranoside.

The known compounds **3**−**7** were identified on the basis of 2D NMR data and comparison of the NMR data with published data.[26,30] Echitaminic acid (**3**) and echitamidine *N*-oxide (**4**) have been isolated from the stem bark of *Alstonia glaucescens* collected in Songkhla, Thailand,[26]

while N^b-demethylalstogustine *N*-oxide (**5**) and N^b-demethylalstogustine (**7**) have been isolated from the stem bark of *Alstonia angustifolia* collected in Bogor (Java, Indonesia).[30] Akuammicine *N*-oxide (**6**) has been isolated as a minor alkaloid from the root bark of *A. scholaris* collected in Thailand in 1976 and was identified by chemical correlation with akuammicine.[19] In 1989, compound **6** was reisolated from the stem bark of *A. angustifolia* and was identified by high-resolution ^{1}H NMR spectroscopy.[30]

We also evaluated the published NMR data for **3**−**7**. Previous NMR data for **3** and **4** were recorded in DMSO-d_6.[26] In this paper we now report NMR data for **3** and **4** recorded in MeOH-d_4, which may assist in the identification of these compounds. The published ^{13}C NMR assignments[30] for some carbons of **5** and **7** have been revised since our NMR data showed that the assignments for the quaternary carbons C-2 and C-8 and for the aromatic carbons C-9 and C-11 should be exchanged. This is the first report of ^{13}C NMR data for **6**. For compounds **5**−**7**, full proton assignments are given here for the first time. Finally, all the compounds reported here were tested for in vitro antimalarial activities. Compounds **6** and **7** had IC_{50} values of 63.2 and 6.75 μg/mL, respectively, against *Plasmodium falciparum* (K1, multidrug-resistance strain). To the best of our knowledge, antimalarial activities have previously not been reported for these compounds.

The heartwood bark of specimens of *A. scholaris* collected in the Philippines contains echitamine as the major component, together with echitamine derivatives, tubotaiwine, and angustilobine-type alkaloids as the minor components.[14] Echitamine was also isolated as the major component from the root bark of a Thai specimen, together with akuammigine, akuammicine, tubotaiwine, and echitamidine-type alkaloids as the minor components.[19] From the root bark of an Indian speciment, echitamine alone was isolated as the major component.[17] In the current study, echitamine was not isolated from the Indonesian specimen of *A. scholaris*. Instead, echitaminic acid, akuammicine, an akuammigine derivative, and echitamidine-type alkaloids were isolated.

In conclusion, this study provides evidence for the more widespread occurrence of compounds **3**−**5** and **7** in the genus *Alstonia*. It also helps to confirm that there is biogeographic variation in the types of indole alkaloids isolated from the bark of *A. scholaris* collected in India, Thailand, the Philippines, and Indonesia. However, compared to the alkaloids isolated from the leaves of *A. scholaris*,[13] the bark alkaloids showed less structural variation.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. UV spectra were recorded on a Cary 50 UV−vis spectrophotometer. ^{1}H, ^{13}C, HSQC, HMBC, NOESY, ROESY, and DQF-COSY spectra were recorded on a Bruker AMX 400 MHz or a Bruker DRX 500 NMR MHz spectrometer. ^{1}H NMR spectra were referenced relative to $CDCl_3$ (δ = 7.26 ppm) or MeOH-d_4 (δ = 3.30 ppm). ^{13}C NMR spectra were referenced relative to $CDCl_3$ (δ = 77.0 ppm) or MeOH-d_4 (δ = 49.0 ppm). HRESIMS was measured on a Finnigan MAT 900 XL double-focusing magnetic sector mass spectrometer. ESIMS was measured on a Micromass ES-TOF LCT quadrupole electrospray time-of-flight mass spectrometer. Flash chromatography was carried out using Scharlau silica gel 60 (0.04−0.06 mm). RP-HPLC was carried out using a Phenomenex Synergi Hydro-RP C_{18} column (250 × 10 mm, 4 μm).

Plant Material. Trunk bark from *Alstonia scholaris* was collected in Kupang, Timor, Indonesia, in April 2003 and air-dried. The plant was identified at the Bogoriense Herbarium in Bogor, Indonesia, by Dr. Irawati. Voucher specimens (AS017) are kept at the IMB, University of Queensland, Brisbane, Australia.

Table 1. ^{13}C NMR Spectral Data for Compounds **1**−**7** (100 MHz)[a]

C	1	2	3	4	5	6	7
2	102.7	164.9	101.9	167.6	165.9	163.5	166.6
3	79.6	79.2	71.6	73.2	75.9	78.1	58.9
5	63.3	68.8	64.4	66.0	67.4	68.2	53.0
6	28.6	41.9	43.6	38.9	39.4	41.3	42.3
7	58.9	57.6	63.3	52.5	53.8	54.1	58.1
8	134.8	133.9	133.1	132.3	132.9	133.3	134.2
9	124.7	121.8	128.7	120.6	120.2	121.7	121.0
10	121.9	122.4	121.2	122.7	121.9	122.2	121.4
11	129.7	130.2	129.6	129.3	129.1	129.4	128.3
12	113.0	111.6	111.6	110.5	110.5	110.2	109.8
13	148.6	145.5	148.3	143.0	144.0	142.9	143.7
14	189.9	26.8	33.6	27.2	24.0	27.9	26.6
15	51.4	25.3	38.6	27.4	24.8	28.2	28.5
16	61.5	104.9	55.5	97.3	103.7	102.0	103.1
17	81.9		67.7				
18	16.2	17.7	15.0	19.8	20.3	13.6	20.2
19	127.3	75.8	130.4	67.1	68.6	129.2	70.6
20	132.6	44.2	135.5	41.8	41.8	131.3	44.4
21	69.1	66.2	67.7	61.7	64.5	72.1	47.7
*C*OO$^-$	174.3		179.2				
*C*O$_2$Me		168.2		168.1	167.3	166.9	167.6
CO$_2$*Me*		51.6		52.4	51.6	51.5	51.5
N(4)-Me	55.4		50.2				
1′		102.5					
2′		75.0					
3′		78.1					
4′		71.5					
5′		78.0					
6′		62.5					

[a] Spectra of **1**−**3** were recorded in MeOH-d_4; spectra of **4**−**7** were recorded in $CDCl_3$.

Extraction and Isolation of Compounds. Powdered, dried bark (1 kg) was extracted with 95% EtOH (6 × 3 L) at room temperature and evaporated in vacuo (<40 °C). The crude extract was partitioned between 3% HCl and Et_2O, and the aqueous layer was basified to pH 10 with NaOH and extracted with $CHCl_3$ to give a crude alkaloidal fraction (2.0 g). The crude alkaloidal fraction was partitioned by NP flash chromatography using CH_2Cl_2, EtOAc, and MeOH in order of increasing polarity. The fraction eluting in 100% MeOH (0.2 g) was further purified using semipreparative RP HPLC (H_2O/CH_3CN, 0%−80% CH_3CN in 80 min, flow rate 3 mL/min, UV detector at λ 250 nm) to afford alkaloids **1** (13 mg), **3** (4 mg), **2** (8 mg), **4** (5 mg), **5** (10 mg), **6** (7 mg), and **7** (10 mg) in order of decreasing polarity.

Bioassay. In vitro antimalarial activity was determined by hypoxanthine incorporation techniques as described by Desjardins et al.[31] The inhibitory concentration (IC_{50}) is the concentration that causes 50% reduction in parasite growth compared to the control (parasites growing without any drugs).

Compound 1: light yellow amorphous powder; $[\alpha]^{25}_D$ +23.4° (*c* 0.49, MeOH); UV (MeOH) λ_{max} (log ϵ) 221 (3.70), 232 (3.69), 286 (3.36) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.39 (1H, dd, *J* = 7.6, 1.0 Hz, H-9), 7.10 (1H, dt, *J* = 7.6, 1.0 Hz, H-11), 6.83 (1H, dd, *J* = 7.6, 1.0 Hz, H-12), 6.79 (1H, dt, *J* = 7.6, 1.0 Hz, H-10), 5.79 (1H, br q, *J* = 7.0 Hz, H-19), 4.61 (1H, br d, *J* = 15.9 Hz, H-21α), 4.43 (1H, d, *J* = 7.4 Hz, H-3), 4.31 (1H, br d, *J* = 15.9 Hz, H-21β), 4.13 (1H, ddd, *J* = 17.0, 14.4, 5.6 Hz, H-6α), 4.03 (1H, d, *J* = 7.8 Hz, H-17β), 4.02 (1H, d, *J* = 7.4 Hz, H-15), 3.85 (1H, d, *J* = 7.8 Hz, H-17α), 3.61 (1H, ddd, *J* = 14.3, 5.6, 1.5 Hz, H-5α), 3.34 (3H, s, N^+-CH_3), 3.26 (1H, ddd, *J* = 14.3, 14.3, 4.3 Hz, H-5β), 1.83 (3H, dd, *J* = 7.0, 1.8 Hz, H_3-18), 1.78 (1H, ddd, *J* = 17.0, 4.3, 1.5 Hz, H-6β4); ^{13}C NMR data, see Table 1; HRESIMS *m/z* $[M + H]^+$ 367.1653 (calcd for $C_{21}H_{23}N_2O_4$, 367.1658).

Compound 2: light yellow amorphous powder; $[\alpha]^{25}_D$ −148.4° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 246 (3.35), 293 (3.56),

327 (3.57) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.50 (1H, dd, J = 7.4, 1.0 Hz, H-9), 7.21 (1H, dt, J = 7.6, 1.0 Hz, H-11), 6.96 (1H, dt, J = 7.4, 1.0 Hz, H-10), 6.70 (1H, dd, J = 7.6, 1.0 Hz, H-12), 4.35 (1H, dd, J = 3.3, 2.8 Hz, H-3), 4.30 (1H, d, J = 7.8 Hz, H-1′), 3.91 (1H, dq, J = 6.5, 2.6 Hz, H-19), 3.86 (1H, dd, J = 12.2, 2.2 Hz, H-6a′), 3.84 (1H, d, J = 10 Hz, H-5β, partly overlap with H-6a′), 3.79 (3H, s, OCH_3), 3.75 (1H, dd, J = 13.5, 13.5 Hz, H-21β, partly overlap with H-5α), 3.73 (1H, dd, J = 10 Hz, H-5α, partly overlap with H-21β), 3.68 (1H, dd, J = 12.2, 5.7 Hz, H-6b′), 3.65 (1H, dd, J = 13.5, 5.7 Hz, H-21α), 3.46 (1H, dd, J = 3.3, 2.8 Hz, H-15), 3.36 (1H, t, J = 9.1 Hz, H-3′), 3.31 (1H, t, H-4′, overlap with MeOH-d_4 peak), 3.24 (1H, ddd, J = 9.4, 5.7, 2.2 Hz, H-5′), 3.18 (1H, dd, J = 9.1, 7.8 Hz, H-2′), 2.60 (1H, ddd, J = 14.6, 3.3, 3.3 Hz, H-14a), 2.42 (1H, ddd, J = 13.9, 10.0, 6.7 Hz, H-6β), 2.27 (1H, ddd, J = 13.9, 7.2, 5.7 Hz, H-6α), 2.14 (1H, m, H-20), 1.39 (3H, d, J = 6.5 Hz, H_3-18), 1.30 (1H, ddd, J = 14.6, 2.8, 2.8 Hz, H-14b); ^{13}C NMR data, see Table 1; HRESIMS m/z [M + H]$^+$ 519.2354 (calcd for $C_{26}H_{35}N_2O_9$, 519.2342).

Compound 3: light yellow amorphous powder; $[\alpha]^{25}_D$ −42.3° (*c* 0.11, MeOH); ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.78 (1H, d, J = 7.7 Hz, H-9), 7.06 (1H, dt, J = 7.7, 1.4 Hz, H-11), 6.74 (1H, dt, J = 7.7, 1.4 Hz, H-10), 6.73 (1H, d, J = 7.7 Hz, H-12), 5.80 (1H, br q, J = 7.0 Hz, H-19), 4.56 (1H, m, H-3, overlap with H-21a), 4.55 (1H, m, J = 12.0 Hz, H-21a, overlap with H-3), 4.03 (1H, br d, J = 5.3 Hz, H-15), 3.99 (1H, d, J = 12.0 Hz, H-21b), 3.78 (1H, d, J = 12.0 Hz, H-17a), 3.44 (2H, br. d, J = 8.8 Hz, H_2-5), 3.37 (3H, s, N−CH_3), 3.12 (1H, dd, J = 15.0, 5.9 Hz, H-6a), 3.09 (1H, d, J = 12.0 Hz, H-17b), 2.77 (1H, ddd, J = 14.7, 10.9, 5.3 Hz, H-14a), 1.86 (3H, dd, J = 7.0, 2.1 Hz, H_3-18), 1.64 (1H, ddd, J = 14.7, 5.9, 1.0 Hz, H-14b); ^{13}C NMR data, see Table 1; ESIMS m/z [M + H]$^+$ 371.18 ($C_{21}H_{26}N_2O_4$).

Compound 4: light yellow amorphous powder; $[\alpha]^{25}_D$ −348.1° (*c* 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (1H, br s, NH), 7.46 (1H, d, J = 7.6 Hz, H-9), 7.23 (1H, dt, J = 7.6, 1.2 Hz, H-11), 7.01 (1H, dt, J = 7.6, 1.2 Hz, H-10), 6.89 (1H, d, J = 7.6 Hz, H-12), 5.07 (1H, br s, H-3), 4.41 (1H, br d, J = 11.4 Hz, H-5a), 3.91 (3H, s, OMe), 3.81 (1H, dd, J = 11.4, 7.3 Hz, H-5b), 3.72 (1H, dd, J = 13.0, 4.4 Hz, H-21a), 3.53 (1H, br s, H-15), 3.32 (1H, qd, J = 8.8, 6.2 Hz, H-19), 3.08 (1H, t, J = 13.0 Hz, H-21b), 2.61 (1H, ddd, J = 14.4, 13.0, 7.3 Hz, H-6a), 2.55 (1H, ddd, J = 14.1, 3.5, 2.6 Hz, H-14a), 2.32 (1H, m, H-20), 2.24 (1H, dd, J = 14.4, 7.9 Hz, H-6b), 1.54 (1H, ddd, J = 14.1, 4.1, 2.1 Hz, H-14b), 1.23 (3H, br d, J = 6.2 Hz, H_3-18); ^{13}C NMR, see Table 1; ESIMS m/z [M + H]$^+$ 357.18 ($C_{20}H_{25}N_2O_4$).

Compound 5: light yellow amorphous powder; $[\alpha]^{25}_D$ −228.8° (*c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.68 (1H, br s, NH), 7.30 (1H, d, J = 7.6 Hz, H-9), 7.21 (1H, dt, J = 7.6, 1.2 Hz, H-11), 6.97 (1H, dt, J = 7.6, 1.2 Hz, H-10), 6.86 (1H, d, J = 7.6 Hz, H-12), 4.48 (1H, br s, H-3), 4.18 (1H, qd, J = 6.5, 3.2 Hz, H-19), 3.93 (1H, dd, J = 13.5, 6.9 Hz, H-21a), 3.83 (1H, m, H-5a, partly overlap with OMe), 3.80 (3H, s, OMe), 3.69 (1H, ddd, J = 12.0, 7.3, 3.2 Hz, H-5b), 3.64 (1H, dd, J = 13.5, 4.5 Hz, H-21b), 3.44 (1H, m, H-15), 3.20 (1H, ddd, J = 14.4, 4.8, 3.4 Hz, H-14a), 2.57 (1H, ddd, J = 14.1, 11.2, 7.3 Hz, H-6a), 2.26 (1H, ddd, J = 14.1, 7.9, 3.2 Hz, H-6b), 1.90 (1H, m, H-20), 1.38 (3H, br d, J = 6.5 Hz, H_3-18), 1.29 (1H, br d, J = 14.4 Hz, H-14b); ^{13}C NMR, see Table 1; ESIMS m/z [M + H]$^+$ 357.18 ($C_{20}H_{25}N_2O_4$).

Compound 6: white amorphous powder; $[\alpha]^{25}_D$ −279.5° (*c* 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (1H, br s, NH), 7.60 (1H, d, H-9), 7.23 (1H, dt, J = 7.6, 1.1 Hz, H-11), 6.99 (1H, dt, J = 7.6, 1.1 Hz, H-10), 6.86 (1H, d, J = 7.6 Hz, H-12), 5.67 (1H, br q, J = 7.0 Hz, H-19), 4.54 (1H, br s, H-3), 4.34 (1H, br d, J = 15.0 Hz, H-21a), 4.16 (2H, m, H-5a and H-21b), 4.09 (1H, br s, H-15), 3.87 (1H, dd, J = 12.0, 6.8 Hz, H-5b), 3.83 (3H, s, OMe), 2.84 (1H, ddd, J = 14.7, 3.8 2.1 Hz, H-14a), 2.60 (1H, dt, J = 13.8, 6.8 Hz, H-6a), 2.09 (1H, dd, J = 13.8, 6.8 Hz, H-6b), 1.69 (3H, br d, J = 7.0 Hz, H_3-18), 1.49 (1H, td, J = 14.7, 2.6 Hz, H-14b); ^{13}C NMR, see Table 1; ESIMS m/z [M + H]$^+$ 339.19 ($C_{20}H_{23}N_2O_3$).

Compound 7: white amorphous powder; $[\alpha]^{25}_D$ −266.6° (*c* 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (1H, br s, NH), 7.25 (1H, d, J = 7.6 Hz, H-9), 7.18 (1H, dt, J = 7.6, 1.0 Hz, H-11), 6.93 (1H, dt, J = 7.6, 1.0 Hz, H-10), 6.86 (1H, d, J = 7.6 Hz, H-12), 4.21 (1H, br s, H-3), 3.84 (3H, s, OMe), 3.64 (1H, qd, J = 7.6, 6.5 Hz, H-19), 3.34 (1H, m, H-5a), 3.09−2.95 (3H, m, H-5b, H-15, and H-21a), 2.72 (1H, dd, J = 14.4, 6.2 Hz, H-21b), 2.35 (1H, m, H-6a, overlap with H-14a), 2.31 (1H, m, J = 13.2 Hz, H-14a, overlap with H-6a), 2.07 (1H, td, J = 12.6, 6.3 Hz, H-6b), 1.85 (1H, m, H-20), 1.24 (1H, td, J = 13.2, 2.6 Hz, H-14b), 1.15 (3H, br d, J = 6.5 Hz, H_3-18); ^{13}C NMR, see Table 1; ESIMS m/z [M + H]$^+$ 341.19 ($C_{20}H_{25}N_2O_3$).

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Supporting Information Available: ^{1}H and ^{13} C NMR spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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