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Cespitulactams A, B, and C, three new nitrogen-containing diterpenes from *Cespitularia taeniata* May

Ya-Ching Shen,[a,*] Yun-Sheng Lin,[a] Yao-Haur Kuo[b] and Yuan-Bin Cheng[a]

[a]*Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 804, Taiwan, ROC*
[b]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan, ROC*

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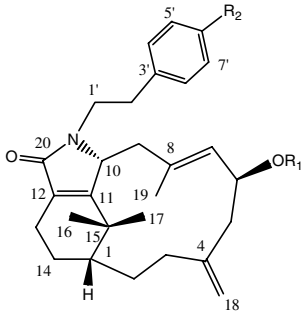
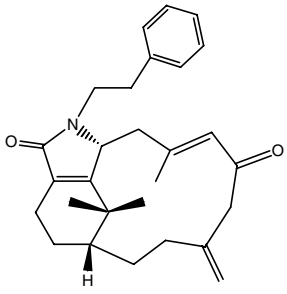
Abstract—Three new nitrogen-containing diterpenoids, designated cespitulactams A (**1**), B (**2**), and C (**3**), were isolated from *Cespitularia taeniata* May. Compounds **1**–**3** are novel structures having a phenylethyl amino side at C-10 and with an amide function at C-20. Their structures were determined on the basis of extensive spectroscopic analysis and chemical correlation. The cytotoxicity of **1** and its monoacetate (**6**) were also evaluated against human cancer cell lines.

The soft corals of the genus *Cespitularia* produce novel secondary metabolites with diverse chemical structures and interesting biological activities.[1–3] These natural products are very close to taxane diterpenes previously isolated from ancient giant trees called yew or *Taxus*.[4,5] The morphology, taxonomy, and ecology of *Cespitularia* spp. are quite different from those of *Taxus* spp. However, their secondary metabolites such as diterpenoids are all derived from the same precursors, geranylgeranyl pyrophosphate and 1S-verticillene.[6] The species of *Cespitularia* habitats as several color variants in the southern coast of Taiwan. In our continuing investigation of bioactive compounds from Taiwanese soft corals,[7–9] we isolated three novel diterpenes designated cespitulactams A (**1**), B (**2**), and C (**3**) having a phenylethyl amino side chain and two known related compounds, cespitularins D (**4**) and F (**5**) from *Cespitularia taeniata* May. In this letter, we reported the isolation, structural elucidation, plausible biogenesis, and cytotoxicity of the new compounds **1**–**3**.

The soft coral (GSCII-16, wet weight 3.5 kg) collected in December, 2003, at a depth of 20 m was extracted with a mixture of CH_2Cl_2 and MeOH, and the extract was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble fraction (20 g) was subjected to Si gel columns (*n*-hexane/acetone, 50:1 to 0:1; *n*-hexane/CH_2Cl_2/MeOH, 100:100:1 to 5:5:1) and HPLC (Si gel, *n*-hexane/CH_2Cl_2/MeOH, 20:115:1; RP-C18, MeOH/H_2O/CH_3CN, 70:25:5) to furnish cespitulactams A (**1**, 25 mg), B (**2**, 3 mg), and C (**3**, 10 mg), cespitularins D (**4**, 8 mg) and F (**5**, 10 mg).[3]



1 $R_1 = R_2 = H$
3 $R_1 = H, R_2 = OH$
6 $R_1 = Ac, R_2 = H$
7 $R_1 = Ac, R_2 = OAc$



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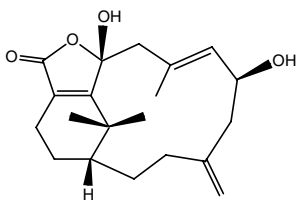

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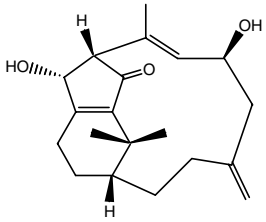

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* Corresponding author. Tel.: +886 7 5252000x5058; fax: +886 7 5255020; e-mail: ycshen@mail.nsysu.edu.tw

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Table 1. ^{1}H and ^{13}C NMR data, HMBC, and COSY correlations of **1**[a]

No.	δ_H (mult, *J*, Hz)	δ_C	HMBC ^{1}H–^{13}C	COSY ^{1}H–^{1}H
1	1.66 (m)	42.9		2, 14
2	1.52 (m, 2H)	18.4		3
3	2.21 (m, 2H)	31.8		2
4		146.6		
5	2.29 (m), 1.52 (m)	43.7	3,4,6,7,18	6
6	4.30 (m)	68.2	4,7,8	7
7	5.30 (d, 7.8)	133.8		6,19
8		133.4		
9	2.51 (m, 2H)	38.2	7,8,10,19	10
10	3.72 (br s)	60.8		9
11		160.4		
12		131.8		
13	2.32 (m), 2.02 (m)	32.0		14
14	2.18 (m), 1.70 (m)	24.1		1,13
15		36.7		
16	1.27 (s, 3H)	25.1	1,11,15	
17	1.03 (s, 3H)	35.0	1,11,15	
18	4.78 (s), 4.80 (s)	113.3	3,4,5	3,5
19	1.36 (s, 3H)	17.0	7,8,9	7
20		170.1		
1′	3.16 (m), 4.20 (m)	41.3	10,20,3′	2′
2′	2.90 (m, 2H)	34.8	3′,4′	1′
3′		139.1		
4′,8′	7.16 (d, 7.0)	128.6	2′,3′	5′
5′,7′	7.25 (t, 7.0)	128.7		4′,6′
6′	7.26 (d, 7.0)	133.8		5′

[a] Data were recorded in $CDCl_3$ on 300 MHz; chemical shifts (δ) and coupling constant are given in parts per million and Hertz, respectively.

Cespitulactam A (**1**),[10] [α] −196 (CH_2Cl_2), was obtained as an amorphous powder and had a molecular formula $C_{28}H_{37}O_2N$, as derived from its HRESIMS at *m/z* 420.2906 ($[M]^+$, calcd 420.2902) indicating 11 degrees of unsaturation. The presence of benzyl, hydroxyl, and lactamyl functions were evidenced by IR absorptions at 3421, 3061, 1712, 1665, 1453 cm^{-1}. The ^{1}H NMR, ^{13}C NMR spectra (Table 1) and DEPT revealed that **1** contained an amide carbonyl (δ_C 170.1), a phenyl group (δ_C 139.1, 133.8, 128.7, 128.6; δ_H 7.16–7.26), six olefinic carbons including four quaternary carbons (δ_C 160.4, 146.6, 133.4, 131.8), a methine carbon and proton (δ_C 133.8; δ_H 5.30, d, $J = 7.8$ Hz) and an exomethylene group (δ_C 113.3; δ_H 4.78s, 4.80s), one aliphatic quaternary carbon (δ_C 36.7), one oxygenated methine carbon and proton (δ_C 68.2, δ_H 4.30), eight methylene carbons (δ_C 43.7, 41.3, 38.2, 34.8, 32.0, 31.8, 24.1, and 18.4), and three methyl groups (δ_C 35.0, 25.1, 17.0; δ_H 1.03, 1.27, 1.36). The corresponding proton and carbon assignments were further determined by COSY and HMQC experiments. The COSY spectrum established connectivities of H-10(δ 3.72)/H-9(δ 2.51)/H-19/H-7/H-6(δ 4.30)/H-5/H-18/H-3/H-2/H-1, and H-13/H-14/H-1 in the verticillene nucleous, and H-1′(δ 3.16, 4.20)/H-2′(δ 2.90) and H-4′(δ 7.16)/H-5′(δ 7.25) in the phenylethyl group as shown in Figure 1. The connection of the partial structures of **1** was verified by HMBC experiment, which confirmed the correlations of H-16/C-1,C-15 and H-17/C-1,C-15 and H-18/C-3,C-4,C-5 and H-6/C-4,C-7, and H-19/C-7,C-8,C-9 as well as correlations of H-1′/C-3′ and H-2′/C-4′ and H-5′/C-3′

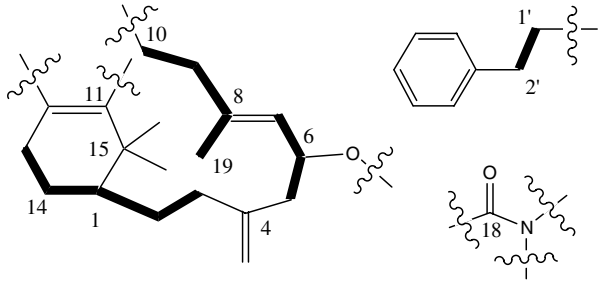


Figure 1. Partial structures of **1** (COSY, ▬).

(Table 1). The above findings account for 8 of the 11 degrees of unsaturation, indicating three additional rings in structure **1**. Acetylation of **1** provided a monoacetate (**6**), which exhibited an acetyl group at δ 2.00.[11] The positions of the phenylethyl and amide groups were determined by observation of HMBC correlations between H-1′ and C-10, and between H-1′ and the amide carbon indicating that **1** contains a novel tricyclic *N*-phenylethyl-butyrolactamyl verticillene system. The relative stereochemistry of cespitulactam A (**1**) was determined by analyses of the NOESY correlations. Assuming that **1** has the same absolute configuration at C-1 as other naturally occurring cespitularines and taxoids, NOESY experiment was performed to ascertain the relative stereochemistries of C-1, H-6, and C-10. The presence of mutual correlations between H-1, H-16, H-17, and H-10 agreed with all β-configurations. The detailed NOESY correlation is illustrated in Figure 2. A computer-modeled structure of **1** was generated by CS Chem 3D version 9.0 using MM2 force field calculation for energy minimization as shown in Figure 3. The result was consistent with the stereochemistry of **1** as established by NOESY experiments. Thus, the structure of **1** was unambiguously established.

Cespitulactam B (**2**), [α] −110 (CH_2Cl_2), possesses the molecular formula $C_{28}H_{35}O_2N$, (2 units less than **1**) as derived from HRESIMS at *m/z* 418.2746 ($[M+H]^+$, calcd 418.2746), indicating 12 degrees of unsaturation.[12] The UV, IR, and ^{1}H NMR spectra of **2** resembled those of **1** suggesting their structural analogy. The ^{1}H NMR

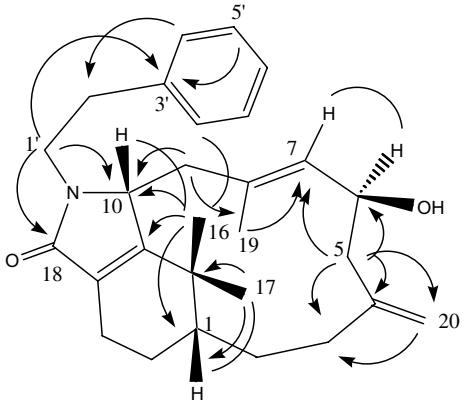


Figure 2. Key NOESY (curve) and HMBC (hook) correlations and relative stereochemistry of **1**.

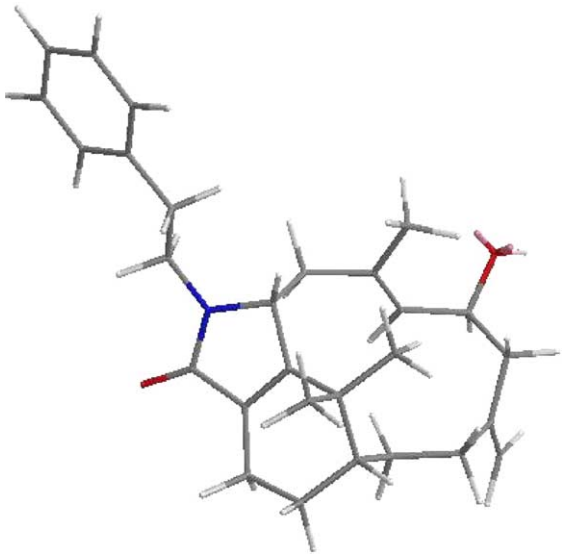
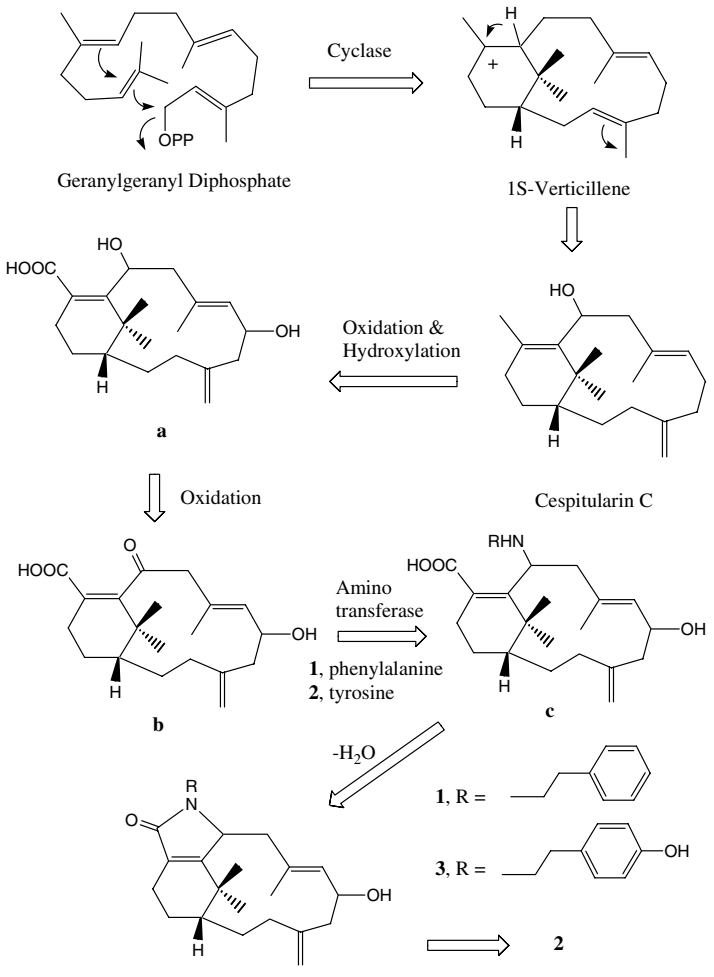


Figure 3. Computer-generated perspective model for **1** using MM2 force field calculation.

spectrum of **2** exhibited characteristic signals including a phenylethyl group (δ 4.21, 3.19, 7.17–7.27), two singlets at δ 4.83 and δ 4.91, and three methyl singlets (δ 1.78, 1.37, 1.05). However, signal of H-6 (δ 4.30) in **1** was missing. Instead, a singlet appeared at δ 6.22 in **2** indicated that the hydroxyl at C-6 in **1** was oxidized to an α,β-conjugated ketone in **2**. The ^{13}C NMR spectrum of **2** showed signals of a conjugated ketone carbonyl (δ 199.0), an amide carbonyl (δ 169.4), six olefinic carbons (δ 160.3, 150.4, 143.8, 135.8, 126.7, and 115.8), three methyl carbons (δ 34.0, 25.2, 16.7), two methine carbons (δ 54.7, 44.8), eight methylene carbons (δ 45.4, 41.2, 38.4, 34.8, 34.2, 29.6, 24.5, 17.6), and a quaternary carbon at 36.9 (C-15). John's oxidation of **1** yielded a product identical with **2**, establishing the structure with its relative stereochemistry.

Cespitulactam C (**3**), [α] −255 (CH_2Cl_2), had the molecular formula $C_{28}H_{37}O_3N$ as determined by HRESIMS at m/z 436.2855 ($[M]^+$, calcd 436.2852) indicating 11 degrees of unsaturation.[13] The UV and IR spectra of **3** were similar to those of **1** and **2**. The ^{1}H and ^{13}C NMR spectra of **3** resembled those of cespitulactam A (**1**) except that **3** contained a *para*-hydroxyphenylethyl side chain (δ_H 6.99, d, $J = 8.4$ Hz, δ_H 6.76, d, $J = 8.4$ Hz; δ_C 155.0s, 130.2s, 129.7d, and 115.6d) rather than a phenylethyl group in **1**. Acetylation of **3** provided



Scheme 1. A plausible biogenetic pathway of **1**–**3**.

an diacetate **7**, which exhibited an aromatic acetyl at δ 2.28, and an aliphatic acetyl at δ 1.99.[14] Other ^{1}H and ^{13}C NMR spectral data of **7** were fully consistent with the assigned structure of cespitulactam C (**3**).

A plausible biogenetic pathway of **1**–**3** was proposed as shown in Scheme 1 based on the recently published cespitularines C, D, and F,[3] and biosynthesis of taxane diterpenes.[15,16] From a biogenetic point of view, geranylgeranyl pyrophosphate and 1S-verticillene are precursors of cespitulactams A–C (**1**–**3**), which might be transformed from cespitularine C through the intermediates **a**, **b**, and **c** via oxidation, hydration, amino transfer, and amide formation. The occurrence of the phenylethylamino and *para*-hydroxyphenylethylamino side chains in **1** and **3** may be explained by incorporation of phenylalanine and tyrosine to the intermediate **b**, respectively.

This letter describes the isolation of three novel diterpenes **1**–**3** from the soft coral *C. taeniata*. Their structures are closely related to 3,8-seco-taxoids,[4,17] but with an unusual *N*-phenylethyl-butyrolactamyl moiety. They all possess strong negative Cotton effects at 235, 244, and 238 nm, respectively, in the CD spectra. Four human cancer cell lines (KB, Hepa, Daoy, and WiDr) were chosen to test compounds **1** and **6** in vitro cytotoxic potentialities. Compound **1** exhibited significant cytotoxicity against human Widr and Daoy cancer cells at IC_{50} 2.72 and 6.34 μg/ml, respectively, while compound **6** was inactive toward four tumor cells (>20 μg/ml). The preliminary biological test revealed that the free hydroxyl function at C-6 in **1** is critical because acetylation of this position leads to a complete loss of activity.

Cytotoxicity assay. The bioassay used against WiDr (human colon adenocarcinoma) and Daoy (human medulloblastoma), KB (humanoral epidermoid carcinoma) and Hepa59T/VGH (human liver carcinoma) tumor cells was based on a MTT assay method. The procedure of assay was carried out as previously described.[18]

Acknowledgments

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10. Amorphous powder, $[\alpha]_D^{25}$ −196 (*c* 1.0, CH_2Cl_2); CD λ_{max} (Mol. CD) 238 (−7.284), 274 (+0.426) nm; UV (MeOH) λ_{max} 217, 230, 257 nm; IR (neat) ν_{max} 3421, 3061, 1712, 1665, 1453, 1266, 1244, 1029, 736, 702 cm^{-1}; EIMS (70 eV) *m/z* 419 ([M]+, 0.1), 420 ($[M+H]^+$, 0.1), 401 (0.1), 402 (0.1), 344 (0.6), 244 (1.4), 105 (53), 91 (100); ESIMS *m/z* 420 $[M+H]^+$; HRESIMS *m/z* 420.2906 (M^+, calcd for $C_{28}H_{38}O_2N$, 420.2902).
11. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (m, H-1), (2H, m, H-2), (2H, m, H-3), (2H, m, H-5), 5.29 (1H, overlap, H-6), 5.29 (1H, s, H-7), (2H, H-9), 3.74 (1H, s, H-10), (1H, m, H-12), 1.90 (1H, m, H-13), (2H, m, H-14), 1.25 (3H, s, H-16), 1.03 (3H, s, H-17), 4.77 (2H, s, H-18), 1.42 (3H, s, H-19), 4.22 (1H, m, H-1′), 3.77 (1H, m, H-1′), 2.89 (2H, m, H-2′), 7.15 (2H, d, $J = 7.5$ Hz), 7.24 (2H, t, $J = 7.5$ Hz), 7.26 (1H, d, $J = 7.5$ Hz); ESIMS *m/z* 462 $[M+H]^+$.
12. Amorphous powder, $[\alpha]_D^{25}$ −110 (*c* 0.05, CH_2Cl_2); CD λ_{max} (Mol. CD) 244 (−4.270), 284 (+0.265) nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.65 (1H, m, H-1), 2.18 (1H, m, H-2), 1.50 (1H, m, H-3), 2.31 (1H, m, H-5), 6.22 (1H, s, H-7), 2.60 (1H, m, H-9), 2.45 (1H, m, H-9), 4.23 (1H, m, H-10), 2.33 (1H, m, H-10), 2.33 (1H, m, H-12), 2.00 (1H, m, H-13), 2.15 (1H, m, H-14), 1.75 (1H, m, H-14), 1.05 (3H, s, H-16), 1.37 (3H, s, H-17), 4.91 (1H, s, H-18), 4.83 (1H, s, H-18), 1.78 (3H, s, H-19), 4.21 (1H, m, H-1′), 3.19 (1H, m, H-1′), 2.92 (2H, m, H-2′), 7.17 (2H, d, $J = 7.5$ Hz, H-4′), 7.26 (2H, t, $J = 7.5$ Hz, H-5′), 7.27 (1H, d, $J = 7.5$ Hz, H-4′); ^{13}C NMR (CD_3OD, 100 MHz): δ 44.8 (d, C-1), 17.6 (t, C-2), 29.6 (s, C-3), 143.8 (s, C-4), 45.4 (t, C-5), 199.0 (s, C-6), 126.7 (d, C-7), 150.4 (s, C-8), 38.4 (t, C-9), 54.7 (d, C-10), 160.3 (s, C-11), 132.0 (s, C-12), 34.2 (t, C-13), 24.5 (t, C-14), 36.9 (s, C-15), 25.2 (q, C-16) 34.0 (q, C-17), 115.8 (t, C-18), 16.7 (q, C-19), 169.4 (s, C-20), 41.2 (t, C-1′), 34.8 (t, C-2′), 139.9 (s, C-3′), 128.6 (d, C-4′, C-8′), 129.2 (d, C-5′, 7′), 134.5 (s, C-6′); ESIMS *m/z* 418.2746 ($[M+H]^+$); HRESIMS *m/z* 418.2746 ($[M+H]^+$, calcd for $C_{28}H_{37}O_2N$, 417.2668).
13. Amorphous powder, $[\alpha]_D^{25}$ −255 (*c* 0.2, CH_2Cl_2); CD λ_{max} (Mol. CD) 235 (−10.885), 272 (+0.250) nm; UV (MeOH) λ_{max} 224, 279 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.68 (m, H-1), 1.54 (2H, m, H-2), 2.22 (2H, m, H-3), 2.30 (1H, m, H-5), 1.53 (1H, m, H-5), 4.31 (1H, m, H-6), 5.34 (1H, d, $J = 7.8$ Hz, H-7), 2.56 (2H, m, H-9), 3.91 (br s, H-10), 2.32 (1H, m, H-13), 2.02 (1H, m, H-13), 2.20 (1H, m, H-14), 1.72 (1H, m, H-14), 1.32 (3H, s, H-16), 1.07 (3H, s, H-17), 4.79 (1H, s, H-18), 4.81 (1H, s, H-18), 1.36 (3H, s, H-19), 3.17 (1H, m, H-1′), 4.19 (1H, m, H-1′), 2.82 (2H, m, H-2′), 6.99 (2H, d, $J = 8.4$ Hz, H-4′, 8′), 6.76 (2H, d, $J = 8.4$ Hz, H-5′, 7′); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 43.0 (d, C-1), 18.3 (t, C-2), 31.8 (s, C-3), 146.6 (s, C-4), 43.7 (t, C-5), 68.3 (d, C-6), 133.8 (d, C-7), 133.4 (s, C-8), 38.2 (t, C-9), 60.7 (d, C-10), 160.4 (s, C-11), 131.8 (s, C-12), 32.1 (t, C-13), 24.2 (t, C-14), 36.8 (s, C-15), 25.1 (q, C-16) 35.1 (q, C-17), 113.5 (t, C-18), 17.0 (q, C-19), 170.1 (s, C-20), 41.3 (t, C-1′), 34.0 (t, C-2′), 130.2 (s, C-3′), 129.7 (d, C-4′, C-8′), 115.6 (d, C-5′, 7′), 155.0 (s, C-6′); ESIMS *m/z* 458 $[M+Na]^+$; 436 $[M+H]^+$; HREIMS *m/z* 436.2855 ($[M+H]^+$, calcd for $C_{28}H_{38}O_3N$, 436.2852).
14. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.67 (m, H-1), 1.50 (2H, m, H-2), 2.21 (2H, m, H-3), 2.28 (1H, m, H-5), 4.31 (1H,

m, H-6), 5.29 (1H, overlap, H-7), 2.56 (2H, m, H-9), 3.75 (br s, H-10), 2.35 (1H, m, H-13), 2.05 (1H, m, H-13), 2.19 (1H, m, H-14), 1.67 (1H, m, H-14), 1.30 (3H, s, H-16), 1.04 (3H, s, H-17), 4.77 (1H, s, H-18), 4.76 (1H, s, H-18), 1.41 (3H, s, H-19), 3.16 (1H, m, H-1′), 4.20 (1H, m, H-1′), 2.89 (2H, m, H-2′), 7.16 (2H, d, $J = 8.4$ Hz, H-4′, 8′), 6.97 (2H, d, $J = 8.4$ Hz, H-5′, 7′), 1.99, 2.28 (s, OAc); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 43.0 (d, C-1), 18.4 (t, C-2), 31.8 (s, C-3), 145.8 (s, C-4), 40.6 (t, C-5), 71.5 (d, C-6), 135.7 (d, C-7), 136.7 (s, C-8), 38.2 (t, C-9), 60.7 (d, C-10), 160.3 (s, C-11), 131.9 (s, C-12), 31.9 (t, C-13), 24.0 (t, C-14), 36.6 (s, C-15), 25.0 (q, C-16) 34.9 (q, C-17), 114.0 (t, C-18), 17.0 (q, C-19), 170.9 (s, C-20), 41.1 (t, C-1′), 34.2 (t, C-2′), 129.6 (s, C-3′), 129.5 (d, C-4′, C-8′), 121.7 (d, C-5′, 7′), 149.3 (s, C-6′), 21.1, 21.2 (q, OCO*C*H$_3$), 169.4, 169.9 (s, O*C*OCH$_3$).

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