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Cytotoxic Lignans from Fruits of *Cleistanthus indochinensis*: Synthesis of Cleistantoxin Derivatives

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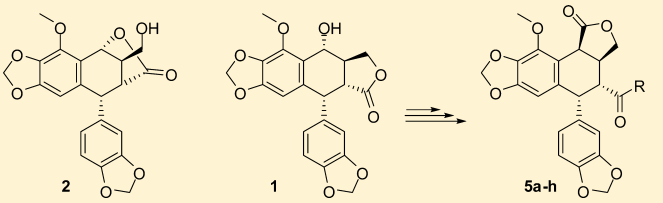
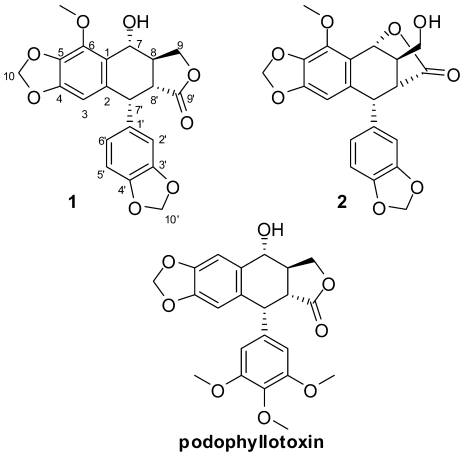
Supporting Information

ABSTRACT: Two new aryl-tetralin lignans, **1** and **2**, were isolated from the fruits of *Cleistanthus indochinensis* by bioassay-guided purification. Their structures were determined by spectroscopic analysis including MS and 2D NMR. The absolute configurations of **1** and **2** were established from examination of their CD spectra. Compound **1** was cytotoxic against KB cells with an IC_{50} value of 0.022 μM, while compound **2** had weaker cytotoxicity, with an IC_{50} value of 1.4 μM. When tested against other cancer cell lines (MCF-7, MCF-7R, and HT29), **1** showed an IC_{50} of 0.014 against MCF-7R cells and an IC_{50} of 0.036 μM against MCF-7 cells. A series of amide derivatives of a new lactone, homoderivatives of **1**, were prepared. Of these derivatives, only compound **3** had weak cytotoxicity against KB cells.



The genus *Cleistanthus* comprises about 140 species and is known for its lignan and terpenoid content.[1−8] Lignan lactones from several species of this genus have structural similarity to podophyllotoxin and its semisynthetic anticancer derivatives such as etopoide and teniposide.[1,9,10] In a previous study, we reported the isolation of a new triterpenoid skeleton from the leaves of *Cleistanthus indochinensis*.[11] In our screening program, a fruit extract of *C. indochinensis* Merr. ex Croiz (Euphorbiaceae) showed strong cytotoxicity against KB cells (>90% inhibition at 1 μg/mL). In this paper, we report the isolation and structural elucidation of two new cytotoxic lignans, **1** and **2**, from the fruits of *C. indochinensis*. Compound **1** was a principle active component of the fruits against KB, MCF-7, MCF-7R, and HT9 cell lines. The structure of **1** is similar to that of podophyllotoxin, which has been found to epimerize easily at C-7 in acidic medium and at C-8′ in basic solution. The epimerization of podophyllotoxins at C-7 affords epipodophyllotonxins that change their anticancer properties (inhibition of microtubule polymerization vs topoisomerase II inhibition),[12,13] while the epimerization at C-8′ in many cases reduces their cytotoxicity.[14,15] A number of stable podophyllotoxin derivatives with lactone ring-opening have been reported, and some of them could be promising new antitumor drug candidates.[16−18] In order to obtain more stable derivatives of **1**, a series of a new type of related lignans were prepared for evaluation of their biological activities.



RESULTS AND DISCUSSION

Dried and ground fruits of *C. indochinensis* (0.5 kg) were extracted with CH_2Cl_2 at room temperature. The solvent was removed under reduced pressure to give a residue of 28.5 g. This crude extract showed strong inhibition against KB cells and then was chromatographed by open column chromatography (CC). The resulting fractions were retested against KB cells, and the two active fractions were further purified by CC to give compounds **1** (2.1 g) and **2** (0.25 g).

Compound **1** was isolated as a white, microcrystalline solid. Its HRESI mass spectrum showed the pseudomolecular ion $[M + Na]^+$ consistent with the molecular formula $C_{21}H_{18}O_8$. The ^1H NMR spectrum of **1** presented a singlet aromatic proton at δ_H 6.22 (H-3) and an ABX ring system, characterized from three aromatic protons at δ_H 6.72 (H-2′), 6.66 (H-5′), and 6.64 (H-6′). The presence of a methoxy and two methylendioxy

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Table 1. NMR Data for 1 and 2 ($CDCl_3$, ^1H: 500 MHz, ^{13}C: 125 MHz)

1				2			
position	δ_C, type	δ_H	mult. (J in Hz)	position	δ_C	δ_H	mult. (J in Hz)
1	124.7, C			1	119.7		
2	132.9, C			2	131.6		
3	104.1, CH	6.22	s	3	104.4	6.20	s
4	149.4, C			4	150.1		
5	134.9, C			5	135.2		
6	141.5, C			6	140.5		
7	70.4, CH	4.99	d (9.0)	7	72.1	5.81	br d (5.0)
8	38.7, CH	2.84	dddd (9.0, 9.0, 10.5, 15.0)	8	46.6	3.13	m
9	71.8, CH_2	4.02, Hβ	dd (9.0, 10.5)	9	59.4	3.62	br dd (8.0, 10.5)
		4.59, Hα	dd (9.0, 9.0)			3.71	br dd (7.5, 10.5)
10	101.2, CH_2	5.88	d (1.5)	10	101.3	5.92	d (1.0)
		5.91	d (1.5)			5.93	d (1.0)
1′	133.0, C			1′	135.2		
2′	111.1, CH	6.72	d (1.5)	2′	109.8	6.49	br s
3′	147.2, C			3′	147.7		
4′	146.5, C			4′	147.0		
5′	107.6, CH	6.66	d (8.0)	5′	108.2	6.73	d (9.0)
6′	124.1, CH	6.64	dd (1.5, 8.0)	6′	122.7	6.56	br d (9.0)
7′	43.9, CH	4.47	d (4.5)	7′	43.2	4.24	d (4.5)
8′	44.6, CH	2.71	dd (4.5, 15.0)	8′	47.2	2.94	ddd (1.0, 4.5, 4.5)
9′	174.2, C			9′	175.7		
10′	100.9, CH_2	5.86	d (1.3)	10′	101.1	5.93	br s
		5.87	d (1.3)				
OMe	59.8, CH_3	4.12	s	OMe	60.3	4.04	s

groups was also noted, together with six protons in the aliphatic region. The ^{13}C NMR and DEPT spectra showed signals of 12 aromatic carbons, a carboxylic group, four sp^3 methines, a sp^3 methylene, a methoxy, and two dioxymethylenes. The chemical shifts of C-4, C-5, C-6, C-3′, and C-4′ (Table 1) were characteristic of oxygenated aromatic carbons. Similarly, the ^1H and ^{13}C chemical shifts of CH-7 and CH_2-9 suggested their linkage to oxygen. Analysis of the $^1H-^1H$ COSY spectrum revealed two spin−spin coupling systems: (a) correlations of the ABX system; (b) cross-peaks of H-8 (δ_H 2.84) to H-7 (δ_H 4.99), CH_2-9 (δ_H 4.02 and 4.59), and H-8′ (δ_H 2.71), which was further correlated to H-7′ (δ_H 4.47). These analyses suggested that **1** was an aryl-tetralin lignan, which was supported by HMBC analysis. Cross-peaks of C-7′ (δ_C 43.9) with H-3, H-2′, and H-6′ indicated linkage of C-7′ to both the A- and B-rings. Correlation of C-1 (δ_C 124.7) with H-8 and that of C-6 (δ_C 141.5) to H-7 assigned the C-1−C-7 connection. The lactone ring was revealed from HMBC cross-peaks of the carboxylate carbon C-9′ (δ_C 174.2) to CH_2-9 protons. The dioxymethylene protons at δ_H 5.88 and 5.91 were correlated to C-4 (δ_C 149.4) and C-5 (δ_C 134.9), indicating the position of this dioxymethylene group at C-4 and C-5 of the B-ring. Similarly, bonding of the dioxymethylene at δ_H 5.86 and 5.87 to C-3′ and C-4′ was established from their HMBC correlations. Finally, C-6 was correlated to the methoxy signal at δ_H 4.12 that defined the presence of an OCH_3 group at C-6.

The relative configuration of **1** was deduced from $^1H-^1H$ coupling constant analysis. H-7 presented an *anti* coupling constant (J = 9.0 Hz), assigning its pseudodiaxial relationship with H-8. H-8′ was observed as a doublet of doublets in the ^1H NMR spectrum and had both *gauche* (J = 4.5 Hz) and *anti* (J = 15.0 Hz) coupling constants. This indicated that H-8′ had a pseudoaxial orientation, while H-7′ was pseudoequatorial. This observation was confirmed by a NOESY experiment in which interaction of H-7 and H-8′ was noted.

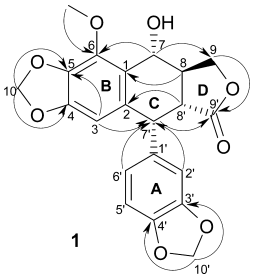

Figure 1. Selected HMBC correlations for **1**.

The absolute configuration (7′*R*) was established by examination of the circular dichroism (CD) spectrum of **1**, which gave a positive Cotton effect at 295 nm ($\Delta\varepsilon$ +2.16). Since the sign of the first couplet is determined by the configuration of the aryl substituent at C-7′, namely, negative for 7′*S* and positive for 7′*R*, C-7′ was assigned the *R*-configuration.[19−21] On the basis of the relative configuration determined above, the *R*-configuration was assigned for the remaining chiral centers, C-7, C-8, and C-8′. Compound **1** was thus identified as (7*R*,8*R*,7′*R*,8′*R*)-7-hydroxy-6-methoxy-3′,4′:4,5-bis(methylenedioxy)-2,7′-cyclolignan-9′,9-olide. This is the first report of a compound with this structure, and compound **1** has been named cleistantoxin. The structure of **1** is similar to that of podophyllotoxin, which is well known for its antiviral and antitumor properties.[22]

The ^{13}C NMR spectrum of compound **2** presented signals similar to those of **1**. However, their ^1H NMR spectra were remarkably different, especially in the aliphatic region. The $^1H-^1H$ COSY spectrum analysis indicated the same spin−spin coupling systems as **1**, and analysis of the HMBC spectrum of **2**

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indicated the same A-, B-, and C-ring systems as in the structure of **1**. However, cross-peaks of carboxylate C-9′ (δ_C 175.7) with H-7 (δ_H 5.81) in the HMBC spectrum of **2** indicated that the lactone ring was formed from the carbonyl C-9′ and the OH group at C-7. Further HMBC analysis defined the planar structure of **2** (Figure 2).

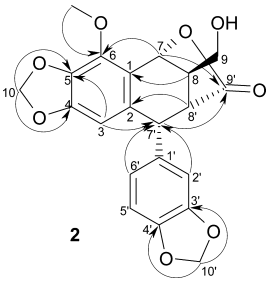


Figure 2. Key HMBC correlations for **2**.

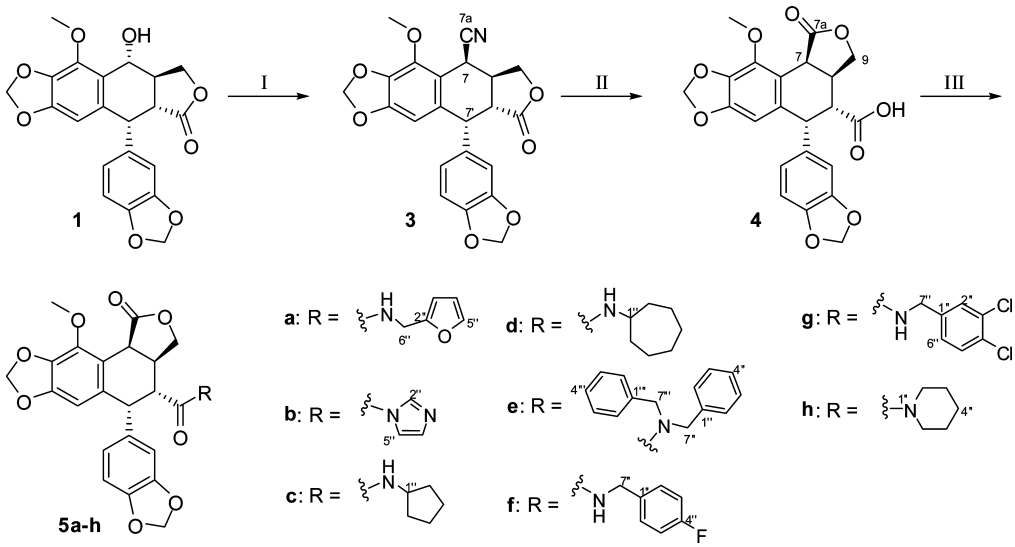
The relative configuration of **2** was also established from proton coupling constant analysis and a NOESY experiment. H-7′ (δ 4.24) was strongly correlated with H-8′ (δ 2.94) and CH_2-9 (δ 3.62 and 3.71) in the NOESY spectrum, which indicated that H-7′, H-8′, and CH_2-9 were on the same face of the C-ring. H-7′ and CH_2-9 were assigned as being pseudoaxial. The positive Cotton effects at 293 nm ($\Delta\varepsilon$ +6.2) in the CD spectrum of **2** indicated the *R*-configuration for C-7′.[19−21] The absolute configuration of **2** was thus identical to that of **1**. Compound **2** was identified as (7*R*,8*R*,7′*R*,8′*R*)-9-hydroxy-6-methoxy-3′,4′:4,5-bis(methylenedioxy)-2,7′-cyclolignan-9′,7-olide and was named neocleistantoxin.

Lignans **1** and **2** were evaluated for their cytotoxicity. Cleistantoxin (**1**) had strong activity against KB cells with an IC_{50} value of 0.022 μM. Compound **2** had moderate cytotoxicity (IC_{50}: 1.4 μM). Cleistantoxin (**1**) also had significant activity against MCF-7 (IC_{50} 0.036 μM), MCF-7R (IC_{50} 0.014 μM), and HT29 (IC_{50} 0.035 μM) cancer cell lines. Compounds **4** and **5a**−**h** were inactive against KB cells.

Cleistantoxin (**1**) is the major active component from the fruits of *C. indochinensis*. It is easily epimerized at C-7 in acidic medium and at C-8′ in basic solution, as observed for podophyllotoxin. In order to obtain a small library of stable derivatives of **1** for evaluation of their biological properties, a series of amide derivatives with a C−C bond at C-7 instead of a C−O linkage, and with lactone ring modification, were prepared as shown in Scheme 1. Accordingly, compound **1** was treated with Me_3SiCN in the presence of $InCl_3$ and Me_3SiBr in CH_2Cl_2 at reflux,[23] providing compound **3** in 64% yield. Configuration inversion at C-7 was observed for **3**, as indicated by the small coupling constant of H-7 (J = 5.5 Hz). Exposure of **3** to a dioxane/aqueous 2 N HCl mixture at 70 °C for 6 h afforded **4**. The HMBC experiment indicated the presence of a new lactone ring by correlations of the carboxylate carbon C-7a (δ 175.2) to H-7 (δ 4.39) and CH_2-9 (δ 4.15 and 4.53). The structure of **4** was then confirmed by X-ray diffraction (Figure 3), in which the configuration inversion at C-7 was clearly observed. Due to the highest anomalous contribution from oxygen atoms,[24] the absolute configuration of 7*S*,8*S*,7′*R*,8′*R* was assigned for **4**, which was in agreement with the CD spectrum analysis of **1**. The formation of the new lactone ring in **4** could be explained by the Pinner reaction followed by hydrolysis.[25] Compound **4** was converted into the acid chloride with oxalyl chloride and then treated with different amines in CH_2Cl_2 at room temperature to afford amides **5a**−**h**. These compounds were found to be more stable in alkaline solutions, such as pyridine, than cleistantoxin (**1**). It is important to note that cleistantoxin (**1**) is easily epimerized at C-8′ in pyridine or DBU at room temperature.

All of the derivatives (**5a**−**h**) were tested against KB cells. Loss of cytotoxicity was observed for all of these synthetic derivatives in comparison with cleistantoxin (**1**). The most active derivative was **3** (IC_{50} 1.2 μg/mL), followed by **5d** (IC_{50} 26 μg/mL) and **5h** (IC_{50} 42 μg/mL). The other compounds were noncytotoxic at a concentration of 100 μg/mL. However, this is the first synthesis of aryl-tetralin lignan derivatives with a lactone ring between C-9 and the carboxylate carbon attached

Scheme 1[a]



[a](I) Me_3SiCN, $InCl_3$, Me_3SiBr, CH_2Cl_2, reflux, 5 h, 64%; (II) 2 N HCl, dioxane, 70 °C, 6 h, 70%; (III) 1. $(COCl)_2$, $CHCl_3$, reflux, 3 h; 2. amines, Et_3N, CH_2Cl_2, 25 °C, 40 −75% for two steps.

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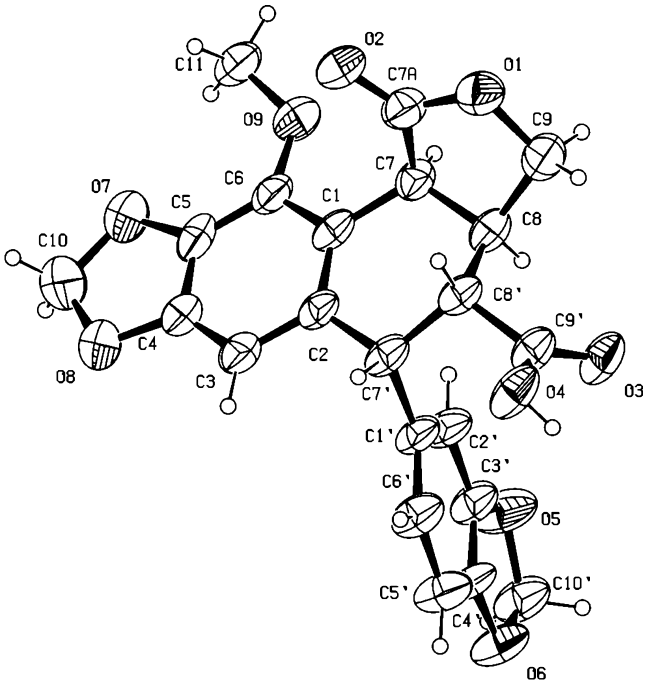

Figure 3. X-ray crystal structure with 30% probability ellipsoids for **4**.

to C-7. This new class of lignan-like compounds should be subjected to further biological screening.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were recorded on a Buchi B-545 instrument and are uncorrected. Optical rotations were recorded on a Polax-2 L polarimeter in $CHCl_3$. UV spectra were recorded on an UV-1601 spectrometer. CD spectra were measured on a JASCO J-810 spectrophotometer. IR spectra were measured on a Nicolet Impact-410 FT-IR spectrometer. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer operating at 500.13 and 125.76 MHz for ^{1}H NMR and ^{13}C NMR spectra, respectively. ^{1}H chemical shifts were referenced to $CDCl_3$ at 7.27 ppm, and ^{13}C chemical shifts were referenced to the central peak of $CDCl_3$ at 77.0 ppm. The HMBC measurements were optimized to 7.0 Hz long-range couplings, and NOESY experiments were run with a 150 ms mixing time. High-resolution ESIMS were measured on a Varian 910 spectrometer.

Plant Material. Fruit of *C. indochinensis* was collected in Quy Chau, Nghe An, Vietnam, on May 6, 2003. The plant material was identified by Dr. Nguyen Quoc Binh, and a voucher specimen (VN-1086) has been deposited at Herbarium of Institute of Ecology and Biological Resources of the Vietnam Academy of Science and Technology, Hanoi, Vietnam.

Extraction and Isolation. Dried and ground fruits (0.5 kg) of *C. indochinensis* were extracted with CH_2Cl_2 at room temperature (3 L × 5 times) for one day each time. The CH_2Cl_2 solution was concentrated under reduced pressure to dryness, and the residue (28.5 g) was chromatographed on a silica gel column, eluted with mixtures of CH_2Cl_2/MeOH (0 to 100% CH_2Cl_2 in MeOH), to give 15 fractions, which were tested against KB cells. Two active fractions (7 and 8) were combined (25.1 g) and subjected to CC on silica gel, using a stepwise gradient of acetone in CH_2Cl_2, to give **1** (2.1 g) and **2** (250 mg).

*Cleistantoxin (**1**):* white, microcrystalline solid; mp 195−196 °C (acetone); $[\alpha]^{30}_D$ −148.0 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3565, 3466, 2931, 1773, 1617, 1483, 1297, 1230, 1142, 1047 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.3 (3.39), 240.0 (4.18), 286.7 (3.83); NMR data see Table 1; HRESIMS (positive mode) *m*/*z* 421.0888 $[M + Na]^+$ (calcd 421.0899 for $C_{21}H_{18}NaO_8$).

*Neocleistantoxin (**2**):* white powder; mp 225−226 °C; $[\alpha]^{30}_D$ −44.4 (*c* 0.225, $CHCl_3$); IR (KBr): IR ν_{max} 3447, 2923, 1753, 1621, 1483, 1377, 1242, 1084, 1040 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 212.3 (3.40), 240.0 (4.23), 286.7 (3.82); NMR data see Table 1; HRESIMS (positive mode) *m*/*z* 421.0897 $[M + Na]^+$ (calcd 421.0899 for $C_{21}H_{18}NaO_8$).

*Compound **3**.* To a solution of **1** (1.0 g, 2.51 mmol) in dry CH_2Cl_2 (15 mL) was added $InCl_3$ (157 mg, 0.71 mmol), Me_3SiCN (0.2 mL, 2.51 mmol), and Me_3SiBr (0.38 g, 2.51 mmol). The mixture was heated at reflux for 5 h and then cooled to room temperature. Water (40 mL) was added, and the resulting mixture was extracted with CH_2Cl_2 (3 × 40 mL). The CH_2Cl_2 extracts were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by CC on silica gel, eluted with a mixture of CH_2Cl_2/MeOH (99.5/0.5) to afford **3** (650 mg, 64%): white powder; mp 173−174 °C; $[\alpha]^{30}_D$ −7.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 6.67 (1H, d, *J* = 8.5 Hz, H-5′), 6.53 (1H, d, *J* = 1.5 Hz, H-2′), 6.52 (1H, dd, *J* = 1.5 and 8.5 Hz, H-6′), 6.25 (1H, s, H-3), 5.95 (2H, br s, CH_2-10), 5.90 (1H, d, *J* = 1.5, H_b-10′), 5.89 (1H, d, *J* = 1.5 Hz, H_a-10′), 4.60 (1H, d, *J* = 5.2 Hz, H-7′), 4.47 (1H, dd, *J* = 7.5 and 9.0 Hz, H_b-9), 4.37 (1H, d, *J* = 5.5 Hz, H-7), 4.34 (1H, dd, *J* = 9.0 and 10.5 Hz, H_a-9), 4.17 (3H, s, OMe-6), 3.03 (1H, dd, *J* = 5.2 and 14.0 Hz, H-8′), 2.89 (1H, m, H-8); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 172.8 (C, C-9′), 150.5 (C, C-4), 147.5 (C, C-3′), 146.9 (C, C-4′), 140.5 (C, C-6), 135.1 (C, C-5), 133.0 (C, C-1′), 132.7 (C, C-2), 124.1 (CH, C-6′), 117.6 (C, C-1), 115.3 (C, C-7a), 110.9 (CH, C-2′), 107.8 (CH, C-5′), 104.7 (CH, C-3), 101.6 (CH_2, C-10), 101.1 (CH_2, C-10′), 68.8 (CH_2, C-9), 59.7 (CH_3, OMe-6), 43.4 (CH, C-8′), 42.9 (CH, C-7′), 32.8 (CH, C-8), 28.7 (CH, C-7); HRESIMS (positive mode) *m*/*z* 408.1085 $[M + H]^+$ (calcd 408.1083 for $C_{22}H_{18}NO_7$).

*Compound **4**.* To a solution of **3** (600 mg, 1.47 mmol) in dioxane (10 mL) was added 2 N HCl (10 mL). The mixture was heated at 70 °C for 6 h and cooled to room temperature. EtOAc (100 mL) was added, and the aqueous layer was removed. The EtOAc solution was washed with 5% $NaHCO_3$ (2 × 20 mL), then with water (50 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column (5−20% MeOH in CH_2Cl_2) to give **4** (440 mg, 70%): white powder; mp 234−235 °C; $[\alpha]^{30}_D$ −10.6 (*c* 1.8, $CHCl_3$); IR ν_{max} 3468, 2930, 1778, 1629, 1481, 1388, 1242, 1115, 1048 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.5 (3.21), 229.7 (4.04), 287.7 (3.61); ^{1}H NMR (500.13 MHz, CD_3COCD_3) δ 6.67 (1H, d, *J* = 8.0 Hz, H-5′), 6.53 (1H, d, *J* = 2.0 and 8.0 Hz, H-6′), 6.46 (1H, d, *J* = 2.0 Hz, H-2′), 6.26 (1H, s, H-3), 5.98 (1H, d, *J* = 1.0 Hz, H_b-10), 5.97 (1H, d, *J* = 1.0 Hz, H_a-10), 5.92 (1H, d, *J* = 1.0 Hz, H_b-10′), 5.90 (1H, d, *J* = 1.0 Hz, H_a-10′), 4.53 (1H, dd, *J* = 5.5 and 9.5 Hz, H_b-9), 4.52 (1H, d, *J* = 5.5 Hz, H-7′), 4.39 (1H, d, *J* = 7.0 Hz, H-7), 4.15 (1H, d, *J* = 9.5 Hz, H_a-9), 4.06 (3H, s, OMe-6), 3.08 (1H, ddd, *J* = 5.5, 7.0, and 13.5 Hz, H-8), 2.95 (1H, dd, *J* = 5.5 and 13.5 Hz, H-8′); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 175.2 (C, C-7a), 172.9 (C, C-9′), 149.9 (C, C-4), 148.3 (C, C-3′), 147.4 (C, C-4′), 142.7 (C, C-6), 136.6 (C, C-5), 135.9 (C, C-1′), 132.7 (C, C-2), 123.3 (CH, C-6′), 116.7 (C, C-1), 110.2 (CH, C-2′), 108.4 (CH, C-5′), 104.1 (CH, C-3), 102.1 (CH_2, C-10), 101.9 (CH_2, C-10′), 70.5 (CH_2, C-9), 59.9 (CH_3, OMe-6), 46.9 (CH, C-7′), 45.8 (CH, C-8′), 39.9 (CH, C-7), 33.0 (CH, C-8); HRESIMS (positive mode) *m*/*z* 427.1025 $[M + H]^+$ (calcd 427.1029 for $C_{22}H_{19}O_9$).

General Procedure for Synthesis of 5a−h. Compound **4** (30 mg, 0.07 mmol) was dissolved in $CHCl_3$ (1 mL), and oxalyl chloride (0.5 mL) was added dropwise. The resulting mixture was heated at 60 °C for 4 h and then concentrated under vacuum to dryness. The residue was dissolved in 1 mL of CH_2Cl_2. To this solution were added amines (1.5 equiv) and Et_3N (3 equiv). The mixture was stirred at room temperature for 12 h and then concentrated under reduced pressure. The residues were purified by CC on silica gel using a stepwise gradient of MeOH in CH_2Cl_2, to give amides **5a−h**.

*Compound **5a**:* 40% yield; white powder; mp 198−199 °C; $[\alpha]^{30}_D$ −147.3 (*c* 1.2, $CHCl_3$); IR ν_{max} 3461, 2930, 1773, 1633, 1479, 1248, 1120 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 210.5 (2.52), 233.5 (4.15), 288.1 (3.78); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.43 (1H, br d, *J* = 2.0 Hz, H-5″), 6.57 (1H, d, *J* = 8.0 Hz, H-5′), 6.37 (1H, dd, *J* = 2.0 and

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3.5 Hz, H-4″), 6.25 (1H, br d, J = 3.5 Hz, H-3″), 6.23 (1H, dd, J = 1.5 and 8.0 Hz, H-6′), 6.20 (1H, d, J = 1.5 Hz, H-2′), 6.12 (1H, s, H-3), 5.90 (1H, d, J = 1.5 Hz, H_b-10), 5.89 (1H, d, J = 1.5 Hz, H_a-10), 5.88 (1H, d, J = 1.5 Hz, H_b-10′), 5.87 (1H, d, J = 1.5 Hz, H_a-10′), 4.50, (1H, dd, J = 4.5 and 15.0 Hz, H_b-6″), 4.41 (1H, dd, J = 4.7 and 10.0 Hz, H_b-9), 4.27 (1H, dd, J = 4.5 and 15.0 Hz, H_a-6″), 4.22 (1H, d, J = 7.0 Hz, H-7), 4.19 (1H, d, J = 5.2 Hz, H-7′), 4.11 (3H, s, OMe-6), 4.08 (1H, d, J = 10.0 Hz, H_a-9), 3.10 (1H, ddd, J = 4.7, 7.0, and 12.5 Hz, H-8), 2.78 (1H, dd, J = 5.2 and 12.5 Hz, H-8′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.3 (C, C-7a), 170.5 (C, C-9′), 151.0 (C, C-2″), 149.2 (C, C-4), 147.4 (C, C-3′), 146.5 (C, C-4′), 142.3 (CH, C-5″), 141.5 (C, C-6), 135.6 (C, C-5), 134.1 (C, C-1′), 131.4 (C, C-2), 122.1 (CH, C-6′), 114.9 (C, C-1), 110.6 (CH, C-4″), 109.2 (CH, C-2′), 108.0 (CH, C-3″), 107.9 (CH, C-5′), 103.4 (CH, C-3), 101.1 (CH_2, C-10′), 101.0 (CH_2, C-10), 70.3 (CH_2, C-9), 59.8 (CH_3, OMe-6), 47.6 (CH, C-7′), 46.3 (CH, C-8′), 39.6 (CH, C-7), 36.3 (CH_2, C-6″), 32.3 (CH, C-8); HRESIMS (positive mode) m/z 506.1490 $[M + H]^+$ (calcd 506.1451 for $C_{27}H_{24}NO_9$).

Compound ***5b:*** 60% yield; yellow powder, mp 244−245 °C; $[\alpha]^{30}_D$ −139.0 (c 1.1, $CHCl_3$); IR ν_{max} 3480, 2926, 1778, 1736, 1628, 1472, 1238, 1102, 1049 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 239.0 (4.19), 278.3 (3.81); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 8.24 (1H, br s, H-2″), 7.50 (1H, br s, H-4″), 7.23 (1H, br s, H-5″), 6.57 (1H, d, J = 8.0 Hz, H-5′), 6.13 (1H, s, H-3), 6.01 (1H, d, J = 1.5 Hz, H-2′), 5.93 (1H, dd, J = 1.5 and 8.0 Hz, H-6′), 5.92 (2H, br s, CH_2-10), 5.89 (2H, br s, CH_2-10′), 4.51 (1H, dd, J = 5.0 and 10.5 Hz, H_b-9), 4.41 (1H, d, J = 5.0 Hz, H-7′), 4.34 (1H, d, J = 7.0 Hz, H-7), 4.14 (3H, s, OMe-6), 4.05 (1H, d, J = 10.5 Hz, H_a-9), 3.59 (1H, dd, J = 5.0 and 12.0 Hz, H-8′), 3.32 (1H, ddd, J = 5.0, 7.0, and 12.0 Hz, H-8); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 174.2 (C, C-7a), 168.7 (C, C-9′), 149.6 (C, C-4), 147.9 (C, C-3′), 147.2 (C, C-4′), 141.6 (C, C-6), 136.2 (CH, C-2″), 135.9 (C, C-5), 132.7 (C, C-1′), 132.0 (CH, C-5″), 129.9 (C, C-2), 121.6 (CH, C-6′), 115.9 (CH, C-4″), 114.4 (C, C-1), 108.7 (CH, C-2′), 108.2 (CH, C-5′), 103.3 (CH, C-3), 101.3 (CH_2, C-10′), 101.2 (CH_2, C-10), 69.8 (CH_2, C-9), 59.9 (CH_3, OMe-6), 47.4 (CH, C-7′), 46.2 (CH, C-8′), 39.3 (CH, C-7), 32.2 (CH, C-8); HRESIMS (positive mode) m/z 477.1297 $[M + H]^+$ (calcd 477.1298 for $C_{25}H_{21}N_2O_8$).

Compound ***5c:*** 47% yield; yellow powder; mp 186−187 °C; $[\alpha]^{30}_D$ −97.9 (c 0.62, $CHCl_3$); IR ν_{max} 3454, 2965, 1774, 1643, 1480, 1249, 1156, 1048 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.9 (3.01), 231.8 (4.17), 288.3 (3.75); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.64 (1H, d, J = 8.0 Hz, H-5′), 6.35 (1H, br d, J = 8.0 Hz, H-6′), 6.26 (1H, br s, H-2′), 6.11 (1H, s, H-3), 5.90 (2H, br s, CH_2-10), 5.88 (2H, br s, CH_2-10′), 4.41 (1H, dd, J = 5.2 and 10.0 Hz, H_b-9), 4.21 (1H, d, J = 7.0 Hz, H-7), 4.16 (1H, d, J = 5.2 Hz, H-7′), 4.12 (1H, m, H-1″), 4.11 (3H, s, OMe-6), 4.08 (1H, d, J = 10.0 Hz, H_a-9), 3.08 (1H, ddd, J = 5.2, 7.0, and 12.5 Hz, H-8), 2.68 (1H, dd, J = 5.2 and 12.5 Hz, H-8′), 1.98 (2H, m, H_b-2″ and H_b-5″), 1.71 (2H, m, H_b-3″ and H_b-4″), 1.62 (2H, m, H_a-3″ and H_a-4″), 1.45 (1H, m, H_a-2″) 1.34 (1H, m, H_a-5″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4 (C, C-7a), 170.0 (C, C-9′), 149.2 (C, C-4), 147.5 (C, C-3′), 146.6 (C, C-4′), 141.5 (C, C-6), 135.6 (C, C-5), 134.3 (C, C-1′), 131.5 (C, C-2), 122.1 (CH, C-6′), 114.9 (C, C-1), 109.3 (CH, C-2′), 107.8 (CH, C-5′), 103.3 (CH, C-3), 101.1 (CH_2, C-10), 101.0 (CH_2, C-10′), 70.4 (CH_2, C-9), 59.8 (CH_3, OMe-6), 51.3 (CH, C-1″), 47.7 (CH, C-7′), 46.4 (CH, C-8′), 39.6 (CH, C-7), 32.4 (CH, C-8), 33.0 (CH_2, C-2″ and C-5″), 23.7 (CH_2, C-3″ and C-4″); HRESIMS (positive mode) m/z 494.1817 $[M + H]^+$ (calcd 494.1815 for $C_{27}H_{28}NO_8$).

Compound ***5d:*** 75% yield; yellow powder; mp 147−148 °C; $[\alpha]^{30}_D$ −128.5 (c 1.6, $CHCl_3$); IR ν_{max} 3426, 2930, 1777, 1647, 1481, 1390, 1252, 1049 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 245.9 (4.31), 288.5 (4.37); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.63 (1H, d, J = 8.0 Hz, H-5′), 6.35 (1H, dd, J = 1.5 and 8.0 Hz, H-6′), 6.26 (1H, d, J = 1.5 Hz, H-2′), 6.09 (1H, s, H-3), 5.90 (1H, d, J = 1.5 Hz, H_b-10), 5.89 (1H, d, J = 1.5 Hz, H_a-10), 5.87 (2H, brs, CH_2-10′), 4.38 (1H, dd, J = 5.0 and 9.5 Hz, H_b-9), 4.19 1H, (d, J = 7.0 Hz, H-7), 4.14 (1H, d, J = 5.2 Hz, H-7′), 4.11 (3H, s, OMe-6), 4.06 (1H, d, J = 9.5 Hz, H_a-9), 3.86 (1H, m, H-1″), 3.08 (1H, ddd, J = 5.0, 7.0, and 12.5 Hz, H-8), 2.65 (1H, dd, J = 5.2 and 12.5 Hz, H-8′), 1.92 (1H, m, H_b-2″), 1.84 (1H, m, H_b-7″), 1.63 (2H, m, H_b-3″ and H_b-6″), 1.62 (2H, m, H_b-4″ and H_b-5″), 1.50 (2H, m, H_a-4″ and H_a-5″), 1.49 (2H, m, H_a-3″ and H_a-6″), 1.47 (1H, m, H_a-2″), 1.40 (1H, m, H_a-7″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.4 (C, C-7a), 169.3 (C, C-9′), 149.2 (C, C-4), 147.4 (C, C-3′), 146.6 (C, C-4′), 141.5 (C, C-6), 135.5 (C, C-5), 134.4 (C, C-1′), 131.7 (C, C-2), 122.2 (CH, C-6′), 114.9 (C, C-1), 109.3 (CH, C-2′), 107.8 (CH, C-5′), 103.4 (CH, C-3), 101.1 (CH_2, C-10), 101.0 (CH_2, C-10′), 70.4 (CH_2, C-9), 59.8 (CH_3, OMe-6), 50.6 (CH, C-1″), 47.7 (CH, C-7′), 46.4 (CH, C-8′), 39.6 (CH, C-7), 32.4 (CH, C-8), 35.1 (CH_2, C-7″), 35.0 (CH_2, C-2″), 24.1 (CH_2, C-6″), 24.0 (CH_2, C-3″), 28.2 (CH_2, C-5″), 28.1 (CH_2, C-4″); HRESIMS (positive mode) m/z 522.2125 $[M + H]^+$ (calcd 522.2128 for $C_{29}H_{32}NO_8$).

Compound ***5e:*** 40% yield; yellow powder; mp 91−92 °C; $[\alpha]^{30}_D$ −214.9 (c 1.5, $CHCl_3$); IR ν_{max} 3434, 2897, 1780, 1640, 1479, 1385, 1233, 1113, 1043 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.5 (3.06), 235.2 (4.15), 288.1 (3.78); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.39 (4H, m, H-3″, H-5″, H-3‴ and H-5‴), 7.36 (1H, m, H-4‴), 7.35 (1H, m, H-4″), 7.27 (2H, br d, J = 7.5 Hz, H-2″ and H-6″), 7.16 (2H, br d, J = 7.5 Hz, H-2‴ and H-6‴), 6.57 (1H, d, J = 8.0 Hz, H-5′), 6.20 (1H, d, J = 1.5 Hz, H-2′), 6.15 (1H, dd, J = 1.5 and 8.0 Hz, H-6′), 6.05 (1H, s, H-3), 5.93 (1H, d, J = 1.5 Hz, H_b-10), 5.92 (1H, d, J = 1.5 Hz, H_a-10), 5.88 (1H, d, J = 1.5 Hz, H_b-10′), 5.86 (1H, d, J = 1.5 Hz, H_a-10′), 5.20 (1H, d, J = 14.0 Hz, H_b-7″), 4.87 (1H, d, J = 18.5 Hz, H_b-7‴), 4.47 (1H, dd, J = 4.7 and 10.0 Hz, H_b-9), 4.43 (1H, d, J = 18.5 Hz, H_a-7‴), 4.24 (1H, d, J = 7.0 Hz, H-7), 4.11 (1H, d, J = 10.0 Hz, H_a-9), 4.09 (3H, s, OMe-6), 4.07 (1H, d, J = 4.7 Hz, H-7′), 3.84 (1H, d, J = 14.0 Hz, H_a-7″), 3.31 (1H, ddd, J = 4.7, 7.0, and 12.0 Hz, H-8), 3.21 (1H, dd, J = 4.7 and 12.0 Hz, H-8′); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 174.9 (C, C-7a), 170.9 (C, C-9′), 149.2 (C, C-4), 147.5 (C, C-3′), 146.6 (C, C-4′), 141.7 (C, C-6), 136.9 (C, C-1″), 135.7 (C, C-5 and C-1‴), 133.9 (C, C-1′), 131.4 (C, C-2), 129.7 (CH, C-2″ and C-6″), 129.3 (CH, C-3″ and C-5″), 128.7 (CH, C-3‴ and C-5‴), 128.2 (CH, C-4‴), 127.8 (CH, C-4″), 126.6 (CH, C-2‴ and C-6‴), 122.6 (CH, C-6′), 114.9 (C, C-1), 109.6 (CH, C-2′), 107.8 (CH, C-5′), 103.4 (CH, C-3), 101.1 (CH_2, C-10 and C-10′), 70.4 (CH_2, C-9), 59.9 (CH_3, OMe-6), 49.7 (CH_2, C-7‴), 48.3 (CH_2, C-7″), 46.6 (CH, C-7′), 43.0 (CH, C-8′), 39.8 (CH, C-7), 33.7 (CH, C-8); HRESIMS (positive mode) m/z 606.2129 $[M + H]^+$ (calcd 606.2128 for $C_{36}H_{32}NO_8$).

Compound ***5f:*** 55% yield; yellow powder; mp 150−151 °C; $[\alpha]^{30}_D$ −126.6 (c 0.96, $CHCl_3$); IR ν_{max} 3434, 2904, 1779, 1638, 1479, 1384, 1231, 1156, 1045 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 232.6 (4.18), 288.1 (3.90); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.24 (2H, dd, J = 6.0 and 8.0 Hz, H-2″ and H-6″), 7.04 (2H, t, J = 8.5 Hz, H-3″ and H-5″), 6.55 (1H, d, J = 8.0 Hz, H-5′), 6.19 (1H, br d, J = 8.0 Hz, H-6′), 6.15 (1H, s, H-2′), 6.04 (1H, s, H-3), 5.88 (2H, m, CH_2-10′), 5.87 (2H, m, CH_2-10), 4.39 (1H, m, H_b-9), 4.38 (1H, m, H_b-7″), 4.25 (1H, dd, J = 5.5 and 14.5 Hz, H_a-7′), 4.18 (1H, d, J = 7.0 Hz, H-7), 4.11 (1H, d, J = 5.5 Hz, H-7′), 4.08 (3H, s, OMe-6), 4.05 (1H, d, J = 10.0 Hz, H_a-9), 3.07 (1H, ddd, J = 5.5, 7.0, and 12.5 Hz, H-8), 2.70 (1H, dd, J = 5.5 and 12.5 Hz, H-8′); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 175.5 (C, C-7a), 170.6 (C, C-9′), 161.3−163.2 (C, C-4″), 149.2 (C, C-4), 147.5 (C, C-3′), 146.6 (C, C-4′), 141.7 (C, C-6), 135.5 (C, C-5), 134.1 (C, C-1′), 133.8 (C, C-1″), 131.4 (C, C-2), 130.1 (CH, C-2″ and C-6″), 122.1 (CH, C-6′), 115.5−115.6 (CH, C-3″ and C-5″), 114.7 (C, C-1), 109.2 (CH, C-2′), 107.9 (CH, C-5′), 103.3 (CH, C-3), 101.1 (CH_2, C-10), 101.0 (CH_2, C-10′), 70.4 (CH_2, C-9), 59.8 (CH_3, OMe-6), 47.5 (CH, C-7′), 46.3 (CH, C-8′), 43.0 (CH_2, C-7″), 39.6 (CH, C-7), 32.3 (CH, C-8); HRESIMS (positive mode) m/z 534.1564 $[M + H]^+$ (calcd 534.1564 for $C_{29}H_{25}FNO_8$).

Compound ***5g:*** 75% yield; yellow powder; mp 194−195 °C; $[\alpha]^{30}_D$ −140.1 (c 1.7, $CHCl_3$); IR ν_{max} 3427, 2932, 1774, 1642, 1477, 1388, 1248, 1123, 1046 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.9 (2.86), 233.1 (4.39), 286.8 (3.90); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 7.40 (1H, d, J = 8.5 Hz, H-5″), 7.38 (1H, d, J = 1.5 Hz, H-2″), 7.10 (1H, dd, J = 1.5 and 8.5 Hz, H-6″), 6.55 (1H, d, J = 8.0 Hz, H-5′), 6.17 (1H, br d, J = 8.0 Hz, H-6′), 5.99 (1H, s, H-3), 6.10 (1H, br s, H-2′), 5.88 (2H, m, CH_2-10), 5.87 (2H, m, CH_2-10′), 4.36 (1H, dd, J = 5.0 and 10.0 Hz, H_b-9), 4.30 (1H, dd, J = 5.5 and 14.5 Hz, H_b-7″), 4.20 (1H, dd, J = 6.0 and 14.5 Hz, H_a-7″), 4.19 (1H, d, J = 7.0 Hz, H-7), 4.08

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(1H, d, J = 5.7 Hz, H-7′), 4.07 (3H, s, OMe-6), 4.02 (1H, d, J = 10.0 Hz, H_a-9), 3.07 (1H, ddd, J = 5.0, 7.0, and 13.0 Hz, H-8), 2.63 (1H, dd, J = 5.7 and 13.0 Hz, H-8′); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 176.0 (C, C-7a), 170.9 (C, C-9′), 149.2 (C, C-4), 147.4 (C, C-3′), 146.6 (C, C-4′), 141.4 (C, C-6), 138.4 (C, C-1″), 135.4 (C, C-5), 134.2 (C, C-1′), 132.5 (C, C-3″), 131.5 (C, C-2 and C-4″), 130.5 (CH, C-5″), 130.3 (CH, C-2″), 127.8 (CH, C-6″), 122.0 (CH, C-6′), 114.5 (C, C-1), 109.1 (CH, C-2′), 107.9 (CH, C-5′), 103.3 (CH, C-3), 101.2 (CH_2, C-10), 101.1 (CH_2, C-10′), 70.6 (CH_2, C-9), 59.8 (CH_3, OMe-6), 47.3 (CH, C-7′), 46.1 (CH, C-8′), 39.6 (CH, C-7), 32.3 (CH, C-8), 27.3 (CH_2, C-7″); HRESIMS (positive mode) m/z 584.0879 $[M + H]^+$ (calcd 584.0879 for $C_{29}H_{24}Cl_2NO_8$).

Compound ***5h****:* 55% yield; yellow powder; mp 233−234 °C; $[\alpha]^{30}_D$ −147.4 (c 1.4, $CHCl_3$); IR ν_{max} 3444, 2946, 1778, 1640, 1477, 1387, 1245, 1043 cm^{-1}; UV ($CHCl_3$) λ_{max} nm (log ε) 207.9 (2.64), 238.6 (4.18), 288.1 (3.90); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 6.63 (1H, d, J = 8.0 Hz, H-5′), 6.28 (1H, dd, J = 1.5 and 8.0 Hz, H-6′), 6.22 (1H, d, J = 1.5 Hz, H-2′), 6.16 (1H, s, H-3), 5.91 1H, (d, J = 1.0 Hz, H_a-10), 5.90 (1H, d, J = 1.0 Hz, H_b-10), 5.89 1H, (d, J = 1.5 Hz, H_a-10′), 5.88 (1H, d, J = 1.5 Hz, H_b-10′), 4.43 (1H, dd, J = 5.0 and 10.0 Hz, H_b-9), 4.23 (1H, d, J = 7.0 Hz, H-7), 4.12 (3H, s, OMe-6), 4.11 (1H, d, J = 5.0 Hz, H-7′), 4.02 (1H, d, J = 10.0 Hz, H_a-9), 3.61 (1H, m, H_b-2″), 3.59 (2H, m, CH_2-6′), 3.44 (1H, ddd, J = 4.0, 7.5, and 13.5 Hz, H_a-2′), 3.20 (1H, ddd, J = 5.0, 7.0, and 12.0 Hz, H-8), 3.12 (1H, dd, J = 5.0 and 12.0 Hz, H-8′), 1.72 (1H, m, H_b-5″), 1.71 (2H, m, CH_2-4″), 1.64 (1H, m, H_a-5″), 1.61 (2H, m, CH_2-3″); ^{13}C NMR (125.76 MHz, $CDCl_3$) δ 175.3 (C, C-7a), 168.7 (C, C-9′), 149.2 (C, C-4), 147.4 (C, C-3′), 146.5 (C, C-4′), 141.6 (C, C-6), 135.6 (C, C-5), 134.2 (C, C-1′), 131.3 (C, C-2), 122.0 (CH, C-6′), 115.2 (C, C-1), 109.2 (CH, C-2′), 107.8 (CH, C-5′), 103.4 (CH, C-3), 101.1 (CH_2, C-10), 101.0 (CH_2, C-10′), 70.8 (CH_2, C-9), 59.8 (CH_3, OMe-6), 46.7 (CH_2, C-6″), 46.1 (CH, C-7′), 42.9 (CH_2, C-2″), 42.4 (CH, C-8′), 39.7 (CH, C-7), 33.0 (CH, C-8), 26.9 (CH_2, C-5″), 25.6 (CH_2, C-3″), 24.5 (CH_2, C-4″); HRESIMS (positive mode) m/z 494.1814 $[M + H]^+$ (calcd 494.1815 for $C_{27}H_{28}NO_8$).

X-ray Crystallographic Analysis of Compound 4. X-ray crystallographic data were collected at room temperature (293(2) K) on a Rigaku diffractometer (for additional details see Supporting Information). Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (deposit no. CCDC 883776). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxic Activity Assay. The human KB tumor (oral epidermoid carcinoma) cell line was obtained originally from ATCC (Manassas, VA, USA). KB cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 μg/mL), and gentamicin (10 μg/mL). Stock solutions of compounds were prepared in DMSO/H_2O (1:9), and the cytotoxicity assays were carried out in 96-well microtiter plates against human nasopharynx carcinoma KB cells (3×10^3 cells/mL) using a modification of the published method.[26] After 72 h incubation at 37 °C in air/CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Taxotere was used as a reference compound.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of **1**−**4** and **5a**−**h**; crystallographic information file (CIF) for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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