Hydrolyzable Tannins of Tamaricaceous Plants. III.[1] Hellinoyl- and Macrocyclic-Type Ellagitannins from *Tamarix nilotica*

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Three new hellinoyl-type ellagitannins, nilotinins M4 (**7**), D7 (**8**), and D8 (**9**), and a new macrocyclic-type, nilotinin D9 (**10**), together with eight known tannins, hirtellins B (**2**), C (**11**), and F (**12**), isohirtellin C (**13**), tamarixinin A (**3**), tellimagrandins I and II, and 1,2,6-tri-*O*-galloyl-β-D-glucose (**14**), were isolated from an aqueous acetone extract of *Tamarix nilotica* dried leaves. Nilotinin M4 (**7**) is a monomeric tannin possessing a hellinoyl moiety. The structure of **8** demonstrated replacement of one of the HHDP groups at the glucose core O-4/O-6 in ordinary dimeric tannins with a galloyl moiety at O-6. This is a new structural feature among the tamaricaceous ellagitannins. On the basis of the results, reported spectroscopic assignments for **2**, **3**, and the macrocyclic tannins **11**−**13** were revised. Unusual shifts in the NMR spectra of these macrocyclic tannins are also discussed in relation to their conformations. Several tannins isolated from *T. nilotica* were assessed for possible cytotoxic activity against four human tumor cell lines, and nilotinin D8 (**9**) and hirtellin A (**1**) showed high cytotoxic effects.

Interest in the ellagitannin constituents of medicinal plants has grown in the past decade as a result of their vast structural diversity. They show marked antiviral, antimicrobial, immunomodulatory, antitumor, and hepatic protective activities, which are largely dependent on the tannin structures.[2−6] Among the different ellagitannin classes, those isolated from tamaricaceous plants have been described as widely varying in structure, including variations in the type of joining moiety and in the mode of attachment between sugar cores in dimeric and oligomeric structures.[7−12] Hirtellins A (**1**) and B (**2**) and tamarixinin A (**3**) isolated from *Tamarix* and *Reaumuria* species exhibit significant host-mediated antitumor activities against sarcoma 180 in mice.[7,8] Significant stimulation of peripheral blood monocyte iodination has also been reported for *Reaumuria* species tannins, including hirtellin E (**4**) and remurins A (**5**) and B (**6**) (Figure 1).[13] During our continuous investigation to discover new drug candidates from natural sources, we recently identified several compounds belonging to this tannin class in *Tamarix nilotica* (Ehrenb.) Bunge (Tamaricaceae).[1,14] In this study, we present the isolation and identification of 12 additional monomeric and dimeric tannins from this plant. Because cytotoxic drugs play a major role in cancer chemotherapy,[15] we also investigated the cytotoxic effect of several isolated tannins against the human oral tumor cell lines human squamous cell carcinoma (HSC-2, HSC-3, and HSC-4) and human promyelocytic leukemia (HL-60) cells.

Results and Discussion

An aqueous acetone extract of *T. nilotica* dried leaves was subjected to Diaion HP 20 column chromatography. The eluate with H_2O/MeOH (6:4, v/v) was submitted to a combination of chromatographic steps on Toyopearl HW-40, Sephadex LH-20, and MCI-gel CHP-20P gels, followed mainly by HPLC purification to furnish three new hellinoyl-type tannins, nilotinins M4 (**7**), D7 (**8**), and D8 (**9**), and one macrocyclic type, nilotinin D9 (**10**). Additionally, eight known tannins, hirtellins B (**2**),[7] C (**11**),[9] and F (**12**),[9] isohirtellin C (**13**),[9] tamarixinin A (**3**),[8] tellimagrandins I[16] and II,[16] and 1,2,6-tri-*O*-galloyl-β-D-glucose (**14**),[17] were isolated for the first time from *T. nilotica*. These known tannins were identified by spectroscopic evidence and/or comparison of spectroscopic data with those in the literature. Spectroscopic assignments for **2**, **3**, **11**, **12**, and **13** were revised. Furthermore, the ^{13}C NMR assignments of **3**, **11**, **12**, and **13**, which were not reported previously, are shown here.

Structural Elucidation of Hellinoyl-Type Ellagitannins. Nilotinin M4 (**7**) was isolated as an off-white, amorphous powder. Its molecular formula was $C_{48}H_{32}O_{31}$ from the results of the elemental analysis. Prominent ion peaks were at *m/z* 1127 ([M + Na]$^+$), 1122 ([M + NH$_4$]$^+$), and 1105 ([M + H]$^+$) in the ESIMS spectrum. The aromatic region in the ^1H NMR spectrum of **7** displayed two mutually coupled doublets [δ_H 7.09, 6.10 (each 1H, d, $J = 2.4$ Hz)] and a pair of one-proton singlets (δ_H 7.64, 6.74), which are characteristic of the hellinoyl moiety.[7] The region also showed a two-proton singlet (δ_H 6.89) and a pair of one-proton singlets (δ_H 6.60, 6.46), which are diagnostic for galloyl and hexahydroxydiphenoyl (HHDP) groups, respectively.[1,14] The aliphatic region of the spectrum showed seven sets of well-resolved signals (δ_H 5.83−3.82) assignable to protons from a fully *O*-acylated glucose core (Table 1).[1,14] The large coupling constants ($J_{1,2}$ = 8.4 Hz, $J_{2,3} = J_{3,4} = J_{4,5}$ = 9.9 Hz) of these proton signals indicated the existence of a glucose core in the pyranose form with a $^4C_1$ conformation and the acyl group at the anomeric carbon in the β-orientation. The ^{13}C NMR spectrum of **7** showed the aliphatic, aromatic, and carboxylic carbon signals (Tables 2 and 3). These signals were assigned on the basis of correlations in the HSQC and HMBC spectra and corresponded to the structural moieties of **7**. The acylation of the glucose core O-4/O-6 by an HHDP group was suggested by the large chemical shift difference ($\Delta\delta_H$ 1.47) between the geminal coupled signals of the glucose core H-6.[18] It was substantiated by HMBC correlations between HHDP proton signals (δ_H 6.60 and 6.46) and H-6 (δ_H 5.29) and H-4 (δ_H 5.10) signals of the glucose core through common carbonyl carbon signals (δ_C 168.2 and 167.6), respectively (Figure 2). A galloyl group was placed at O-3 of the glucose core, similarly substantiated by an HMBC correlation between the galloyl proton signal (δ_H 6.89) and the glucose H-3 signal (δ_H 5.75) through a common carbonyl signal (δ_C 167.0). Consequently, two galloyl parts of the hellinoyl moiety

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Table 1. ^{1}H NMR Data δ_H (J in Hz) for the Glucose Protons of **2**, **3**, and **7−13** (600 MHz, acetone-d_6/D_2O, 9:1)

	7	8	9	2	3 α-anomer	3 β-anomer	10[a]	11[a]	12[a]	13
glucose-1										
1		6.14, d (8.4)	6.19, d (8.4)	6.16, d (8.4)	5.51, d (3.6)	5.15, d (7.8)	6.01, br s	6.16, br s	6.17, br s	5.04, d (9.0)
2		5.52, dd (8.4,9.9)	5.65, dd (8.4, 9.9)	5.66 dd (8.4, 9.9)	5.24, dd (3.6, 9.9)	5.26, dd (7.8, 9.6)	5.32, dd (7.8, 9.6)	5.51, t (8.7)	5.50, t (8.7)	5.27 t (9.3)
3		5.69, t (9.9)	5.78, t (9.9)	5.79, t (9.9)	5.81, t (9.9)	5.56, t (9.6)	5.60, t (9.6)	5.83, t (9.9)	5.83, t (9.9)	5.52, t (9.6)
4		5.21, t (9.9)	5.14, t (9.9)	5.13, t (9.9)	5.05, t (9.9)	5.06, t (9.6)	3.87, t (9.6)	5.11, t (10.9)	5.11, t (9.9)	5.03, t (9.6)
5		4.37, ddd (1.8, 7.2, 9.9)	4.51, dd (6.6, 9.9)	4.52, dd (6.6, 9.9)	4.67, ddd (1.2, 6.6, 9.9)	4.26, dd (6.6, 9.6)	3.74, ddd (2.4, 4.8, 9.6)	4.47, dd (6.6, 9.9)	4.47, dd (6.6, 9.9)	4.22, dd (6.6, 9.6)
6		5.34, dd (7.2, 13.2)	5.31, dd (6.6, 13.8)	5.30, dd (6.6, 13.2)	5.24, dd (6.6, 13.2)	5.26, dd (6.6, 13.2)	3.75, dd (4.8, 12.6)	5.27, dd (6.6, 13.2)	5.28, dd (6.6, 13.2)	5.22, dd (6.6, 13.2)
		4.14, dd (1.8, 13.2)	3.87, d (13.8)	3.86, d (13.2)	3.78, d (1.2, 13.2)	3.88, d (13.2)	3.85, dd (2.4, 12.6)	3.83, d (13.2)	3.80, d (13.2)	3.77, d (13.2)
glucose-2										
1	5.83, d (8.4)	5.62, d (8.4)	5.54, d (8.4)	5.61, d (8.4)	5.78[b], d (8.4)	5.77[b], d (8.4)	5.58, br s	5.59, br s	5.35, br s	5.04, d (9.0)
2	5.43, dd (8.4, 9.9)	5.38, dd (8.4, 9.9)	5.18, dd (8.4, 9.9)	5.36, dd (8.4, 9.9)	5.39[c], dd (8.4, 9.9)	5.42[c], dd (8.4, 9.9)				5.27, t (9.3)
3	5.75, t (9.9)	5.68, t (9.9)	5.49, t (9.9)	5.67, t (9.9)	5.74 t (9.9)		5.70, t (9.9)	5.71, t (9.9)	5.52, t (9.6)	5.52, t (9.6)
4	5.10, t (9.9)	4.03, t (9.9)	3.85, t (9.9)	5.18, t (9.9)	5.09, t (9.9)		5.11, t (9.9)	5.10, t (9.9)	3.84, t (9.6)	5.03, t (9.6)
5	4.43, ddd (1.2, 6.6, 9.9)	4.17, ddd (2.4, 4.8, 9.9)	3.73, ddd (2.4, 5.4, 9.9)	4.35, dd (6.6, 9.9)	4.42, ddd (1.2, 6.6, 9.9)		4.27 dd (6.6, 9.9)	4.28, dd (6.6, 9.9)	3.54, ddd (2.4, 4.8, 9.6)	4.22, dd (6.6, 9.6)
6	5.29, dd (6.6, 13.2)	4.50, dd (4.8, 12.6)	3.88. dd (5.4, 12.6)	5.32, dd (6.6, 13.2)	5.29, dd (6.6, 13.2)		5.22, dd (6.6, 13.2)	5.22, dd (6.6, 13.2)	3.68, dd (4.8, 12.0)	5.22, dd (6.6, 13.2)
	3.82, dd (1.2, 13.2)	4.60, dd (2.4, 12.6)	4.07 dd (2.4, 12.6)	4.14, d (13.2)	3.85, dd (1.2, 13.2)		3.75, d (13.2)	3.74, d (13.2)	3.78, dd (2.4, 12.0)	3.77, d (13.2)

[a] The ^{1}H−^{1}H COSY data for these tannins were obtained from experiments measured at 40 °C. [b,c] Interchangeable.

in **7** were assigned to O-1 and O-2 of the glucose core. This was confirmed by the HMBC correlations of two *meta*-coupled doublets (δ_H 7.09 and 6.10) of the hellinoyl G1-ring and the glucose H-1 proton signal (δ_H 5.83) through a carbonyl signal (δ_C 164.9) and by the correlation of a proton singlet (δ_H 6.74) of the hellinoyl G2-ring with the glucose H-2 signal (δ_H 5.43) through a carbonyl signal (δ_C 163.2). In turn, an HMBC correlation between a proton singlet (δ_H 7.64) of the hellinoyl G3-ring and a carbonyl signal (δ_C 166.5) was found, whereas no correlation of that carbon signal with any of the glucose proton signals was observed. These correlations indicated the orientation of the hellinoyl moiety as shown in formula **7** (Figure 2). The *S* configuration of the HHDP group in **7** was assigned due to a strong positive Cotton effect at 238 nm in the CD spectrum.[19] On the basis of these findings, structure **7** was assigned to nilotinin M4. This is the first example of a monomeric tannin possessing a hellinoyl moiety.

Nilotinin D7 (**8**) was isolated as an off-white, amorphous powder. Its molecular formula, $C_{75}H_{54}O_{48}$, was determined by HRESIMS. The ^{1}H NMR spectrum of **8** showed typical signals from four galloyl groups [δ_H 7.12, 7.03, 6.94, 6.93 (each 2H, s)], an HHDP group [δ_H 6.71 and 6.50 (each 1H, s)], and a hellinoyl group [δ_H 6.79, 5.80 (each 1H, d, J = 2.4 Hz), 7.56, 6.64 (each 1H, s)]. In the higher-field region, two seven-spin aliphatic proton signal systems with large coupling constants (Table 1), which were clearly distinguished from each other by ^{1}H−^{1}H COSY, accounted for two β-glucopyranose cores in the $^4C_1$ conformation. The carbon signals (Tables 2 and 3) seen in the ^{13}C NMR spectrum of **8** were consistent with the presence of these acyl units and the glucose cores. These acyl moieties were placed on the glucose cores on the basis of the following spectroscopic findings. The glucose-2 C-6 methylene proton signals with downfield shifts (δ_H 4.60, dd, J = 2.4, 12.6 Hz, and δ_H 4.50, dd, J = 4.8, 12.6 Hz) showed a small chemical shift difference ($\Delta\delta_H$ ca. 0.1), analogous to that observed for the C-6 methylene proton signals of the glucose core in 1,2,6-tri-*O*-galloyl-β-D-glucose (**14**). Combined with the resonance of the glucose-2 H-4 with an upfield shift [δ_H 4.03 (1H, t, J = 9.9 Hz)], the structure with the HHDP group at O-4/O-6 in ordinary ellagitannins was replaced with a galloyl group at the glucose-2 O-6, and the free OH-4 was assigned. This was further confirmed by an HMBC correlation between the glucose-2 galloyl proton signal (δ_H 7.03) and the H-6 signal (δ_H 4.50) through a common carbonyl carbon signal (δ_C 166.8). In contrast, a large chemical shift difference ($\Delta\delta_H$ 1.20) between the glucose-1 C-6 *gem*-proton signals (δ_H 5.34 and 4.14) indicated bridging of an HHDP group at the glucose-1 O-4/O-6. The remaining hydroxy groups (OH-1−OH-3) in each glucose core were also acylated as indicated by downfield shifts of the corresponding proton signals (Table 1). The HMBC spectrum showed correlations among galloyl proton signals δ_H 6.94, 7.12, and 6.93 (each 2H, s) and glucose proton signals H-1 (δ_H 6.14) and H-3 (δ_H 5.69) of glucose-1 and H-3 (δ_H 5.68) of glucose-2 through the respective carbonyl carbon signals (δ_C 165.0, 167.2, and 167.1). Consequently, the galloyl parts of the hellinoyl moiety should be placed at O-1 and O-2 of glucose-2 and O-2 of glucose-1. This attachment mode of the hellinoyl moiety was also substantiated by the HMBC correlations between two *meta*-coupled proton signals (δ_H 5.80 and 6.79) of the hellinoyl G1-ring and the H-1 signal (δ_H 5.62) of glucose-2 via a carbonyl carbon signal (δ_C 164.8) and a correlation between the proton signal (δ_H 6.64) of the hellinoyl G2-ring and the H-2 signal (δ_H 5.38) of glucose-2 via a carbonyl carbon signal (δ_C 163.5). However, the proton signal (δ_H 7.56) of the hellinoyl G3-ring showed an HMBC correlation with the glucose-1 H-2 signal (δ_H 5.52) through a carbonyl carbon signal (δ_C 164.4). Other HMBC correlations were also consistent with the proposed structure **8** for this tannin; the important ones among them are represented by the arrows in formula **8** (Figure 2). HHDP group atropisomerism was *S* by a strong positive Cotton effect at 232 nm in the CD spectrum. On the basis of these findings, the

Table 2. ^{13}C NMR Data for the Glucose Carbons of **3** and **8−13** (151 MHz, acetone-d_6/D_2O, 9:1)

	7	**8**	**9**	**3**		**10**	**11**	**12**	**13**
				α-anomer	*β*-anomer				
glucose-1									
1		93.1	93.3	91.0	96.2	93.2	93.4	93.4	93.5
2		71.6	71.3	72.2	73.65	71.0	70.8	70.9	69.9
3		74.3	73.1	71.5	73.9[a]	76.1	73.4[b]	73.5	73.7
4		70.7	70.9	71.3[c]	71.4[c]	68.7	70.6	70.5	70.6
5		72.3	72.8	67.0	71.8	78.2	72.7	72.7	72.5
6		63.0	63.1	63.5		61.4	63.0	63.0	62.9
glucose-2									
1	93.7	93.6	93.4	93.7		93.5	93.4	93.1	93.5
2	70.5	70.5	70.7	70.64[d]		71.6	71.5	71.6	69.9
3	73.8	75.8	76.5	73.7[a]	73.8[a]	73.5	73.5[b]	76.2	73.7
4	70.7	69.4	69.1	70.60[d]		70.6	70.6	68.7	70.6
5	72.5	75.4	77.7	72.5		72.6	72.6	78.2	72.5
6	62.9	63.6	61.5	62.8		63.0	63.0	61.3	62.9

[a−d] Interchangeable.

Table 3. ^{13}C NMR Data for the Aromatic Skeleton of the Hellinoyl-Type Tannins **2**, **3**, and **7−9** (151 MHz, acetone-d_6/D_2O, 9:1)

	7	**8**	**9**	**2**	**3** (α and *β* anomers)
galloyl					
1	119.8	119.3, 119.9, 120.5, 121.0	118.8, 119.8, 120.6	119.2, 119.87, 119.93	(119.73, 119.75, 120.14, 120.17)[a]
2/6	110.0 (2C)	109.8, 110.0, 110.1, 110.4 (2C each)	110.0, 110.3, 110.5 (2C each)	110.0, 110.3, 110.4 (2C each)	(110.0, 110.1)[b]
3/5	145.7 (2C)	145.5 (2C), 145.7 (4C), 145.9 (2C)	145.5, 145.6, 145.7 (2C each)	145.5, 145.65, 145.66 (2C each)	(145.48, 145.52, 145.62)[b]
4	139.3	139.0 (2C), 139.3, 139.5	139.1, 139.2, 139.6	139.0, 139.2, 139.6	(138.98, 139.03, 139.24, 139.26)[a]
7	167.0	165.0, 166.8, 167.1, 167.2	165.0, 166.4, 167.3	164.8, 166.4, 167.1	(166.70, 166.76, 166.96, 167.0)[a]
HHDP					
1	115.6	115.7 (2C)	115.7	115.64, 115.73	(115.56, 115.59, 115.63)[a]
1′	115.78		115.8	115.76, 115.79	115.70, 115.73
2, 2′	125.6, 125.9	125.8, 126.0	125.5, 126.0	125.5, 125.7, 126.1 (2C)	(125.5, 125.7, 125.8, 126.1)[b]
3	108.1	108.5	107.85	107.85, 108.4	107.9, 108.1
3′	107.7	107.8	107.77	107.70, 107.79	107.67, 107.72
4, 4′	145.1 (2C)	145.0, 145.1	145.14, 145.16	145.10, 145.13 (2C), 145.16	(145.1)[b]
5	136.3	136.39	136.3	136.26, 136.33	136.2, 136.3
5′	136.5	136.44	136.4	136.44, 136.46	(136.33, 136.35, 136.47)[a]
6, 6′	144.2 (2C)	144.25, 144.32	144.30, 144.33	144.19, 144.25, 144.31, 144.33	(144.19, 144.22, 144.23, 144.24)[b]
7	168.2	168.3	168.3	168.3, 168.4	(168.2, 168.43, 168.48)[a]
7′	167.6	167.7	167.8	167.7, 167.8	(167.66, 167.68, 167.72, 167.76)[a]
hellinoyl					
1	120	119.5	119.8	119.79	(119.77, 119.93)[c]
2	107.9	107.5	107.3	107.2	(107.72, 107.78)[c]
3	148.4	147.7	147.5	147.5	(147.96, 148.0)[c]
4	140.0	140.0	139.5[d]	139.7	(139.88, 139.95)[c]
5	147.4	146.9	146.7	146.9	(147.10, 147.15)[c]
6	111.2	111.7	111.8	111.8	111.4
7	164.9	164.8	164.8	164.7	(164.8, 164.9)[c]
1′	111.1	111.4	111.4	111.2	(110.6, 110.8)[c]
2′	142.6	142.3	142.2	142.3	(142.5, 142.6)[c]
3′	139.8[e]	139.8[f]	139.8[g]	139.8[h]	139.66[i]
4′	139.5	139.5[f]	139.2[d]	139.4	(139.55[i], 139.51[i])[c]
5′	141.8	141.8	141.8	141.8	141.8
6′	108.8	108.7	109.0	108.8	108.8
7′	163.2	163.5	163.4	163.4	163.5
1″	115.82	114.3	114.5	114.6	(113.9, 114.4)[c]
2″	143.44[j]	145.3	144.6	144.6	(143.85, 143.94)[c]
3″	140.2[e]	140.6[f]	140.7[g]	140.6[h]	(140.5, 140.7)[c]
4″	139.7	140.1	139.7	139.75	(139.66[i], 139.75[i])[c]
5″	143.36[j]	143.3	143.2	143.2	(143.17, 143.25)[c]
6″	119.4	118.9	119.0	119.0	(118.6, 118.7)[c]
7″	166.5	164.4	164.0	164.1	163.9

[a] Chemical shifts between each two parentheses equals 2C in total. [b] Chemical shifts between each two parentheses equals 4C in total. [c] Chemical shifts between each two parentheses equals 1C in total. [d−j] Interchangeable.

nilotinin D7 structure was represented by **8**. Replacing the HHDP group at glucose-2 O-4/O-6 with a galloyl group at O-6 is a new structural feature among those of the tamaricaceous plant hydrolyzable tannins. This result suggests a wide structural diversity among the hydrolyzable tannins of these plants.

Nilotinin D8 (**9**) was isolated as an off-white, amorphous powder. Its molecular formula, $C_{68}H_{50}O_{44}$, was established by the elemental analysis results and the spectroscopic data shown below. The ^1H NMR spectrum exhibited proton signals assignable to a hellinoyl group [δ_H 6.78, 5.68 (each 1H, d, $J = 1.8$ Hz), 7.56, 6.68 (each 1H, s)], an HHDP group [δ_H 6.60 and 6.55 (each 1H, s)], three galloyl groups [δ_H 7.00, 6.98, and 6.90 (each 2H, s)], and proton signals from two *β*-glucopyranose cores in the $^4C_1$ conformation (Table 1). The ^{13}C NMR spectroscopic data of **9** (Tables 2 and 3) were consistent with the presence of these constituent units. The ESIMS of **9** showed an ion peak $[M + Na]^+$ at *m/z* 1593, corresponding to the molecular formula shown above. This is the same as that of hirtellin E (**4**), which was also composed of the same constituent units.[9] Although a close resemblance was observed among the proton signals of **9** and those reported for **4**,[9] a large chemical shift difference ($\Delta\delta_H$ 1.44) between the C-6 methylene glucose-1 proton signals (rather than the corresponding one for glucose-2 as in **4**) indicated placement of the HHDP group in **9** at the glucose-1 O-4/O-6. The placements of the acyl groups on the glucose cores in **9** were substantiated by the HMBC correlations among aromatic and glucose proton signals through three-bond couplings with carbonyl carbons, as illustrated by the arrows in formula **9** (Figure 2). The *S* configuration of the HHDP group in **9**

Table 4. ^{13}C NMR Data for the Aromatic Skeleton of the Macrocyclic-Type Tannins **10−13** (151 MHz, acetone-d_6/D_2O, 9:1)

	10[a]	**11**[a]	**12**[a]	**13**
galloyl				
1	120.0, 120.7	119.9, 120.0	120.0, 120.6	119.6 (2C)
2/6	110.0, 110.1 (2C each)	110.0 (4C)	110.0, 110.1 (2C each)	110.1 (4C)
3/5	145.8, 145.9 (2C each)	145.75, 145.76 (2C each)	145.77, 145.82 (2C each)	145.6 (4C)
4	139.2, 139.3	139.31, 139.35	139.2, 139.35	139.3 (2C)
7	166.8, 167.0	166.7, 166.83	166.9, 167.2	166.9 (2C)
HHDP				
1	115.6	115.6 (2C)	115.6	115.5 (2C)
1′	115.9	115.9 (2C)	115.9	115.8 (2C)
2, 2′	125.4, 126.1	125.4 (2C), 126.0, 126.1	125.4, 126.0	125.3, 125.8 (2C each)
3	107.9	107.9 (2C)	107.9	107.9 (2C)
3′	107.7	107.7 (2C)	107.7	107.7 (2C)
4, 4′	145.12, 145.16	145.12, 145.15 (2C each)	145.2 (2C)	145.0, 145.1 (2C each)
5	136.2	136.2, 136.3	136.3	136.2 (2C)
5′	136.5	136.5, 136.6	136.5	136.4 (2C)
6, 6′	144.3 (2C)	144.3 (4C)	144.3 (2C)	144.2 (4C)
7	168.3	168.24, 168.25	168.2	168.3 (2C)
7′	167.8	167.78, 167.82	167.8	167.6 (2C)
DHDG				
1	119.8	119.8	120.0	118.3 (2C)
2	107.4	107.3	107.3	105.5 (2C)
3	147.4	147.4	147.3	147.4 (2C)
4	140.1[b]	140.1[c]	139.9[d]	139.7[e] (2C)
5	146.1	146.2	146.2	146.1 (2C)
6	112.5	112.4	112.3	113.7 (2C)
7	164.1	164.1	164.2	163.8 (2C)
1′	113.7	113.6	113.4	112.2 (2C)
2′	138.0	138.0	138.0	135.0 (2C)
3′	139.0[b]	139.1[c]	139.0[d]	139.7[e] (2C)
4′	141.0[b]	141.2[c]	141.2	140.6[e] (2C)
5′	143.0	143.1	143.2	142.8 (2C)
6′	109.6	109.6	109.6	111.9 (2C)
7′	163.0	162.8	162.9	164.3 (2C)
isoDHDG				
1	123.7	123.4	123.2	
2/6	110.3 (2C)	110.3 (2C)	110.3 (2C)	
3/5	149.0 (2C)	149.1 (2C)	148.5 (2C)	
4	139.1[b]	139.3[c]	139.32[d]	
7	165.1	164.8	164.8	
1′	114.4	114.2	113.9	
2′	139.22[b]	139.13[c]	139.4[d]	
3′	139.6[b]	139.7[c]	139.6[d]	
4′	140.3[b]	140.4[c]	140.5[d]	
5′	142.0	142.0	142.1	
6′	108.5	108.6	108.7	
7′	166.7	166.6	166.5	

[a] The assignments of the carbons of the isoDHDG units in these tannins were achieved by comparison with spectroscopic data of monomeric and dimeric tannins possessing isoDHDG units in our preceding reports.[1,14] [b−e] Interchangeable.

was determined on the basis of a strong positive Cotton effect in the CD spectrum short-wavelength region. Nilotinin D8 was thus formulated as **9**, which is a structural isomer of **4**, with regard to the position of the HHDP group. Noteworthy among the hellinoyl-type tannins **2**, **3** (Figure 1), and **7−9** (Figure 2), the hellinoyl G1-ring H-2 and H-6 signals were distinguished from each other on the basis of the HMBC correlations with the carbon signals at δ_C ~148 and 147 of C-3 and C-5 of the same ring, respectively. In these tannins, the hellinoyl H-2 signals showed upfield shifts (δ_H 5.68−6.10) relative to that of the H-6 signals (δ_H 6.78−7.09) of the same moiety, and the glucose-2 anomeric proton signals also showed noticeable upfield shifts (δ_H 5.54−5.83) relative to that of the corresponding glucose-1 protons (δ_H ~6.2). These shifts were attributable to the anisotropic effects of the hellinoyl G2-rings, as exemplified by formula **9a** shown in Figure 2.

Hirtellin B (**2**) was isolated as the major tannin from the *T. nilotica* aqueous acetone extract. It was first isolated from *Reaumuria hirtella* Jaub *et* Sp. and *T. pakistanica* Quaiser (Tamaricaceae).[7,8] Although structure **2** was established previously, our careful investigation of the ^{1}H−^{1}H COSY spectrum of **2** revealed that the assignments of one of the glucose-1 H-6 signals (δ_H 5.32) and the corresponding glucose-2 H-6 signal (δ_H 5.30) should be interchanged (for corrected proton assignments, see Table 1). Similarly, the HSQC spectrum indicated that the HHDP C-3 (δ_C 108.86) and the hellinoyl C-6′ (δ_C 108.35) carbon signal assignments should be reversed (for corrected carbon assignments, see Table 3). Additionally, the hellinoyl C-3″ signal was not assigned in the previous report.[7] Because the aromatic skeleton ^{13}C NMR signals of **2** were not fully assigned and the ^{1}H NMR signals were not shown in detail in the previous report,[7] spectroscopic experiments including ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, and HMBC (Figure 1) were performed, and the results are summarized in Table 3 (also see Experimental Section).

Tamarixinin A (**3**) was isolated as a major tannin from the *T. nilotica* aqueous acetone extract. It was first isolated from *T. pakistanica* Quaiser.[8] However, the ^{1}H−^{1}H COSY data obtained in our study revealed that the proton signals that were previously assigned to glucose-1 H-2−H-5 in the α-anomer should be interchanged with those assigned to the corresponding protons of the same glucose in the β-anomer (for corrected proton assignments, see Table 1), and the assignment of the glucose-1 methylene proton with a higher-field shift should be revised to δ_H 3.78 (H-6α) and 3.88 (H-6β). The total carbon assignments were also achieved on the basis of the NMR experiments similar to those applied for the hirtellin B carbon assignments. The detailed assignments of the aromatic protons of **3** are also shown in the Experimental Section.

Structural Elucidation of Macrocyclic-Type Ellagitannins. Nilotinin D9 (**10**) was isolated as an off-white, amorphous powder. Its molecular formula, $C_{68}H_{50}O_{44}$, was established by HRESIMS

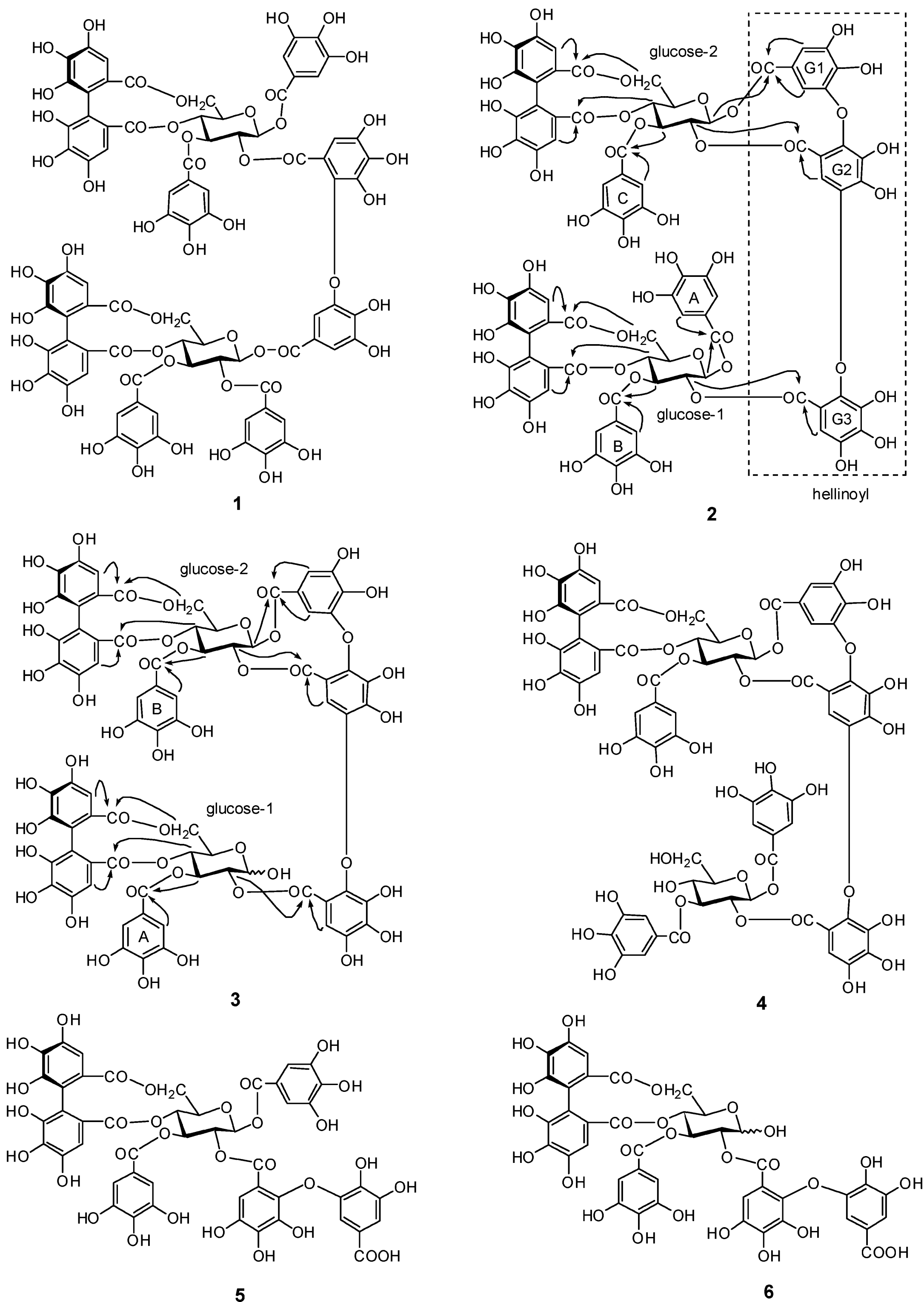

Figure 1. Structures of tannins **1**−**6**. The arrows (H→C) indicate important HMBC correlations.

and the spectroscopic data shown below. The ^{1}H NMR spectrum of **10** showed proton signals diagnostic for an isodehydrodigalloyl (isoDHDG) unit [δ_H 6.84 (2H, br s) and 6.78 (1H, br s)] and a dehydrodigalloyl (DHDG) unit [two *m*-coupled doublets at δ_H 7.10 and 6.44 (each 1H, d, J = 1.8 Hz) and one 1H singlet at δ_H 7.11].[1,9,14] The ^{13}C NMR spectrum also substantiated the presence of these acyl groups (Table 4). Proton signals accounted for the presence of an HHDP group [δ_H 6.58 and 6.48 (each 1H, s)], and two galloyl groups [δ_H 7.05 and 6.97 (each 2H, s)] were also seen in the aromatic region. The spectrum in the higher-field region showed proton signals from two glucose cores (Table 1). Among them, two broad signals [δ_H 6.01 (1H, br s) and δ_H 5.58 (2H, br s)] were assigned to the glucose-1 H-1, and the glucose-2 H-1 and H-2 overlapped with each other on the basis of HSQC correlations. Although the intensity of the 2D cross-peaks for these broad proton signals was low, the larger accumulation of the HSQC data of good-purity tannin permitted their observation. Despite the broadening of the anomeric proton signals, the $^{4}C_{1}$ conformations of the glucose cores were assigned on the basis of the coupling constants of the remaining proton signals (Table 1). The ^{13}C NMR spectrum of **10** showed carbon signals (Tables 2 and 4) consistent with the presence of the galloyl groups and glucose cores. Among the ester carbonyl

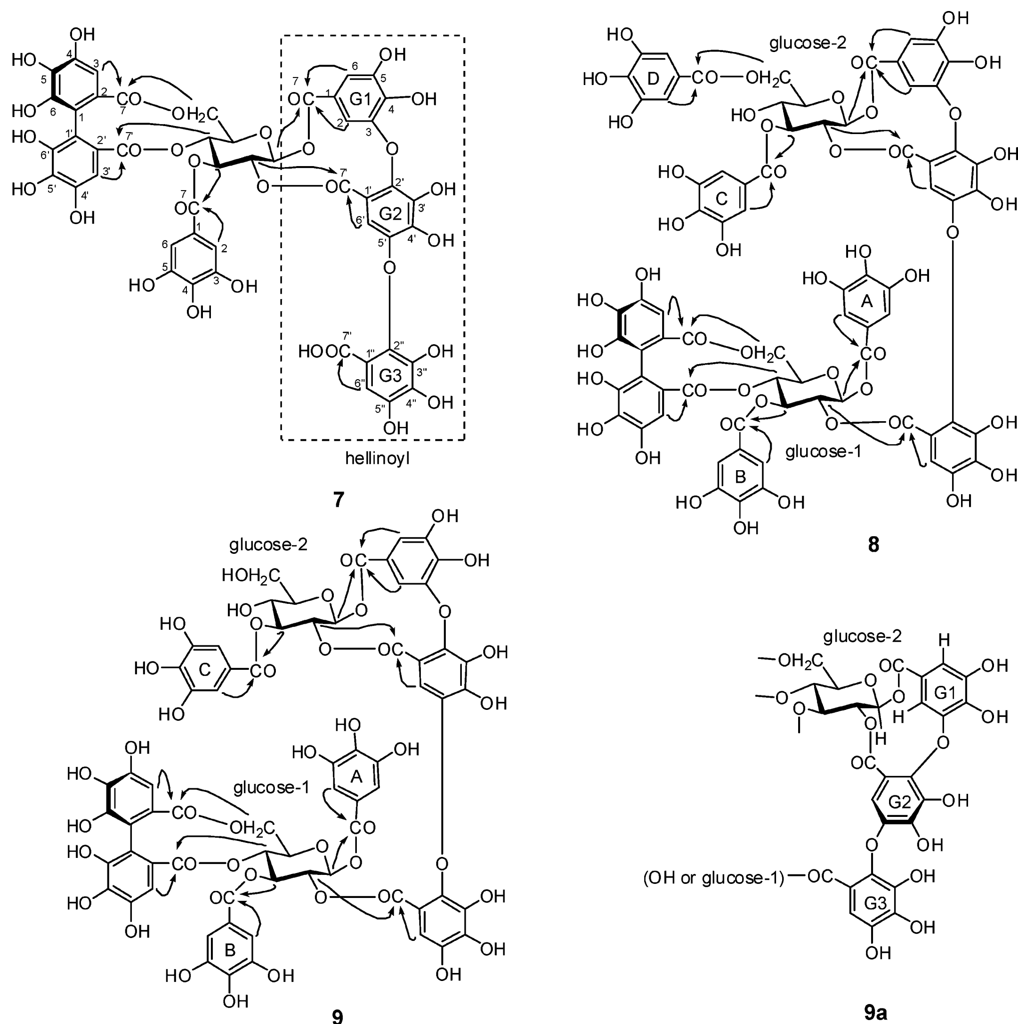

Figure 2. Structures of the new hellinoyl-type tannins **7**−**9** and formula **9a**. The arrows (H→C) indicate important HMBC correlations.

carbons, those assigned to the DHDG (δ_C 163.1 and 164.1) and the isoDHDG (δ_C 165.1 and 166.7) groups showed broad signals, which were attributed to the macrocyclic structure, because analogous features were also seen in those of the related hirtellins C (**11**) and F (**12**).[9] The ^{1}H−^{1}H COSY data for **10** (Table 1) showed upfield shifts in the glucose-1 H-4 (δ_H 3.87) and H-6 (δ_H 3.85 and 3.75), implying the presence of free OH-4 and OH-6 on the glucose-1 pyranose ring. The appearance of the remaining proton signals at lower field (Table 1) indicated complete acylation of the remaining hydroxy groups on the glucopyranose rings. The locations of two galloyl groups were assigned to O-3 of each glucose core on the basis of the HMBC correlations between the galloyl signal at δ_H 7.05 and the glucose-1 H-3 signal (δ_H 5.60) via a common carbonyl carbon signal (δ_C 167.0) and those between the galloyl signal at δ_H 6.97 and the glucose-2 H-3 signal (δ_H 5.70) via a common carbonyl carbon signal (δ_C 166.8). Consequently, each of the DHDG and the isoDHDG groups in **10** were assigned to bridge between the O-1 of one glucose core and the O-2 of the other in a mode corresponding to the formation of a macrocyclic structure. HMBC correlations concerning the broad glucose proton signals due to the glucose-1 H-1, and the glucose-2 H-1 and H-2, with the isoDHDG group protons were not observed. However, the spectrum showed an HMBC correlation between the DHDG H-6′ (δ_H 7.11) signal and the glucose-1 H-2 signal (δ_H 5.32) via a carbonyl carbon peak (δ_C 163.0, DHDG C-7′), indicating that the galloyl part bearing one DHDG unit hydrogen (ring-D2) was attached to the glucose-1 O-2. The remaining carbonyl carbon signal (δ_C 164.1) was assigned to DHDG C-7 on the basis of the two-bond HMBC correlation with the DHDG group H-2 (δ_H 6.44) and H-6 (δ_H 7.10) signals. On the basis of these HMBC observations and the close similarities of the spectroscopic features (see Supporting Information) among **10**, and those of the previously established analogous structures **11** and **12**,[9] the locations and orientations of the DHDG and the isoDHDG units were assigned as shown by formula **10** (Figure 3). The HHDP group in **10** was assigned the *S* configuration by CD spectroscopy. The ESIMS spectrum of **10** exhibited an $[M + Na]^+$ ion peak at *m/z* 1593, which was consistent with the O-4/O-6-des-HHDP hirtellin C (**11**) derivative and also the structural isomer to **12**.[9] On the basis of these findings, structure **10** was assigned to nilotinin D9 (Figure 3).

Broadening of proton and carbon signals is a characteristic spectroscopic feature among oligomeric macrocyclic tannins such as oenotheins A and B, woodfordins C and D, and eugeniflorin D1. It has been explained by the presence of a restricted interconversion among the macro-ring conformations.[20]

Hirtellin C (**11**) was first isolated from *R. hirtella*, and its structure was previously assigned on the basis of chemistry and spectroscopic data, including a ^{1}H−^{13}C long-range COSY experiment for its octacosamethyl derivative.[9] However, despite the identical dimeric nature of hirtellin C (**11**) (Figure 3) and nilotinin D9 (**10**), the reported chemical shift of the glucose-2 anomeric proton signal was much lower (δ_H 6.07, br d)[9] than that of the corresponding signal of **10** (δ_H 5.58, br s). Therefore, we performed intensive NMR spectroscopic experiments including ^{13}C NMR, ^{1}H−^{1}H COSY, HSQC, and HMBC to verify the assignments for **11**. The ^{1}H NMR spectrum of **11** obtained in the present work showed proton signals (Table 1 and Experimental Section) identical to those

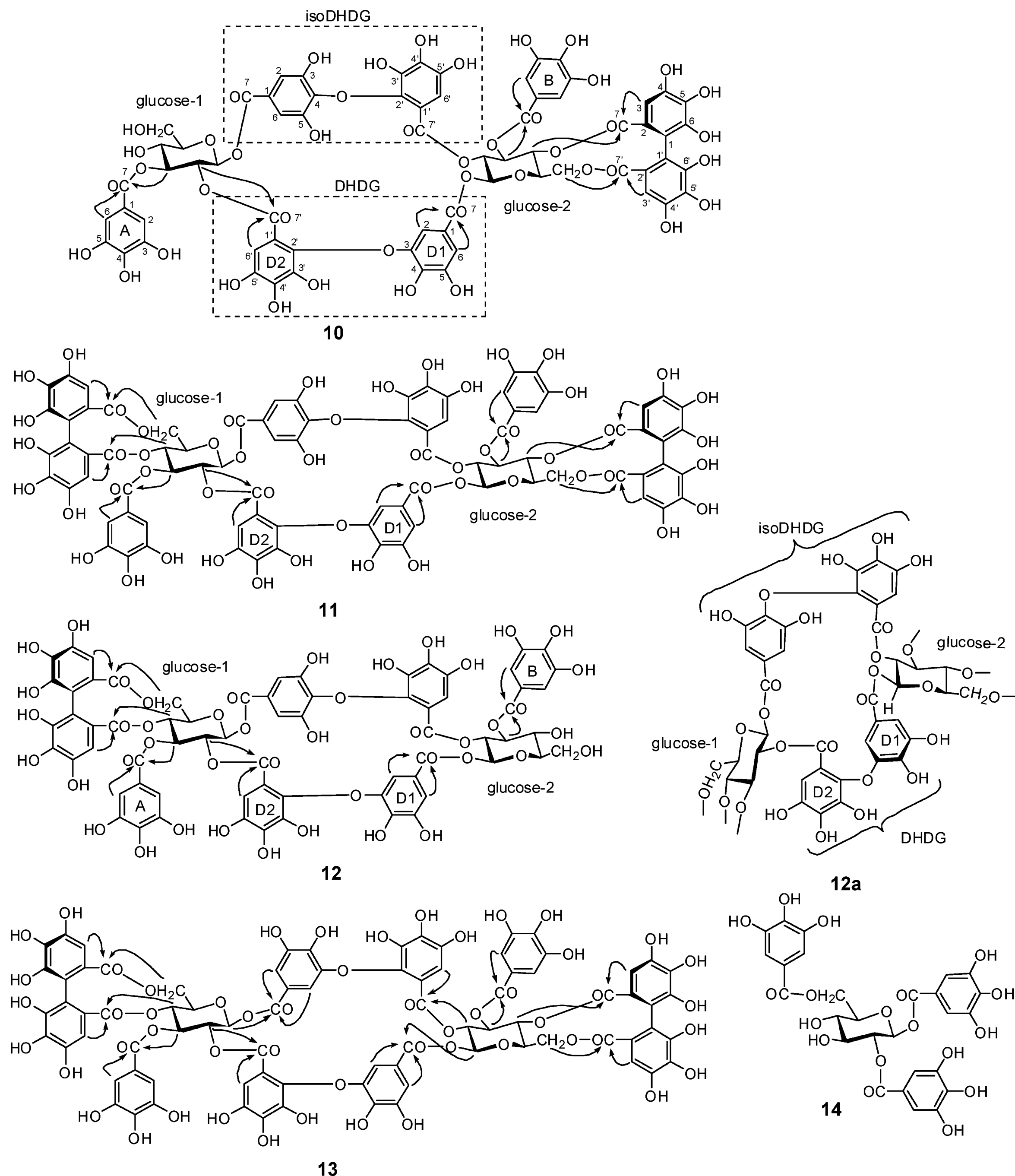

Figure 3. Structures of tannins **10**−**14** and the formula **12a**. The arrows (H→C) indicate important HMBC correlations.

assigned for **11** in a previous report.[9] However, the proton signal at δ_H 6.07, which was previously assigned to the glucose-2 H-1, was not observed, and an alternative proton signal (δ_H 5.59), which was assigned to glucose-2 H-2 in the previous report, was assigned here to two overlapping proton signals of glucose-2 H-1 and H-2 on the basis of its HSQC correlation with two carbon signals (δ_C 93.4 and 71.5). Similarly, the proton signal at δ_H 6.16 (1H, br s) was assigned to the glucose-1 anomeric proton on the basis of the HSQC correlation with the glucose anomeric carbon signal at δ_C 93.4. Because the chemical shift of the signal at δ_H 6.07 in the ^{1}H NMR spectrum of **11** was coincident with the DHDG moiety H-2 signal of isohirtellin C (**13**) (see Experimental Section), this signal was attributed to the contamination with **13**, which could be derived from isomerization of **11**.[9] Analogous problems that were also found when assigning glucose carbons in the previous report[9] have been fixed, and full assignments of the carbon of **11** are reported here (Tables 2 and 4).

Hirtellin F (**12**) was also first isolated from *R. hirtella* (Tamaricaceae), and the macrocyclic structure **12** (Figure 3) was assigned to this tannin.[9] However, the chemical shifts in the previously assigned signals to glucose-1 H-1, glucose-2 H-1 and H-2, and DHDG and the isoDHDG group protons[9] were unusual when compared to those of the related tannins **10** and **11**. In the present study, the ^{1}H NMR spectrum of **12** showed proton signals from two galloyl groups [δ_H 7.04 and 6.94 (each 2H, s)], one HHDP [δ_H 6.58 and 6.48 (each 1H, s)], one isoDHDG [δ_H 6.87 (2H, br s) and 6.83 (1H, br s)], one DHDG [two *m*-coupled doublets at δ_H 6.98 and 6.41 (each 1H, d, J = 1.8 Hz) and one 1H singlet at δ_H 7.12] groups, and two $^4C_1$ glucose cores (Table 1). Among the glucose core proton signals, two broad signals [δ_H 6.17 (1H, br s) and 5.35 (2H, br s)] were assigned analogously to glucose-1 H-1 and overlapped glucose-2 H-1 and H-2 signals, respectively, on the basis of HSQC correlations. The assigned acyl group locations were further substantiated here by the ^{1}H−^{1}H COSY data listed in

Table 5. Cytotoxicity of Tannins **1**−**3**, **5**−**7**, and **9** against Human Normal and Tumor Cells

		CC_{50} (μM)[a] human normal oral cells			promyelocytic leukemia cell		CC_{50} (μM)[a] human squamous carcinoma cells			
	MW	HGF	HPC	HPLF	HL-60	TS_1[b,c]	HSC-2	HSC-3	HSC-4	TS_2[d]
Monomeric Tannins										
remurin A (**5**)	1106	262 ± 3.1	118 ± 20	324 ± 5.0	18.8 ± 4.8	12.5	34.3 ± 0.58	44.7 ± 0.58	84.3 ± 22.4	4.31
remurin B (**6**)	954	177 ± 6.7	78.5 ± 3.5	293 ± 12.5	8.71 ± 0.98	21.0	91.7 ± 36.9	99.3 ± 10.3	74.0 ± 26.5	2.1
nilotinin M4 (**7**)	1104	274 ± 3.1	199 ± 6.8	353 ± 13.3	30.8 ± 2.9	8.9	29.3 ± 2.5	54.3 ± 7.1	59.3 ± 5.0	5.7
Dimeric Tannins										
hirtellin A (**1**)	1874	218 ± 81.1	170 ± 4.6	373 ± 7.4	18.4 ± 0.47	13.8	31.3 ± 3.8	51.0 ± 1.7	48.0 ± 6.9	5.8
hirtellin B (**2**)	1872	337 ± 7.8	255 ± 64.4	375 ± 14.5	41.7 ± 6.6	7.7	32.3 ± 1.2	63.3 ± 3.1	60.0 ± 9.6	6.2
tamarixinin A (**3**)	1720	325 ± 4.0	263 ± 11.2	361 ± 1.2	30.6 ± 4.9	10.3	35.0 ± 1.0	57.0 ± 7.2	62.0 ± 5.6	6.2
nilotinin D8 (**9**)	1570	318 ± 4.9	278 ± 10.9	358 ± 4.0	22.9 ± 2.8	13.9	32.3 ± 2.5	48.3 ± 3.2	54.3 ± 11.0	7.1

[a] Each value represents the mean of at least three independent experiments. [b] TS, tumor specificity. [c] TS_1 = {[CC_{50}(HGF) + CC_{50}(HPC) + CC_{50}(HPLF)]/[CC_{50}(HL-60)]} × 1/3}. [d] TS_2 = {[CC_{50}(HGF) + CC_{50}(HPC) + CC_{50}(HPLF)]/[CC_{50}(HSC-2) + (HSC-3)] + (HSC-4)]}.

Table 1 and the HMBC correlations shown in Figure 3. The full assignment of the carbon atoms of **12** are listed in Tables 2 and 4 for comparison with those of **10** and **11**, and the physicochemical data are shown in the Experimental Section.

Isohirtellin C (**13**) was isolated as an off-white, amorphous powder and identified on the basis of a comparison of its ^{1}H NMR spectroscopic data (see Experimental Section and Table 1) with those in the literature.[9] Although **13** was obtained previously as a hydrolysate of **10**,[9] it was isolated here for the first time from a plant extract. Its ^{13}C NMR assignments (Tables 2 and 4) and the UV, CD, and $[\alpha]_D$ data are shown here.

As for the monomeric and linear dimeric ellagitannins possessing DHDG moieties at O-2 of the glucose cores,[1,8,14] upfield shifts (δ_H ~5.6) of the anomeric protons relative to that of the corresponding protons (δ_H ~6.1) for the other glucose cores are attributable to the shielding effect of the DHDG unit ring-D2. In contrast, among the macrocyclic tannins **10**−**12**, the glucose-1 anomeric protons, with a DHDG moiety ring-D2 at glucose-1 O-2, resonated at low field (δ_H 6.01−6.17), whereas those of glucose-2, with the galloyl part bearing two DHDG moiety hydrogens (ring-D1) at glucose-2 O-1, showed upfield shifts (δ_H ~5.5). These upfield shifts are due to the shielding effect of DHDG moiety ring-D1 and suggested conformations as exemplified by formula **12a** (Figure 3). An analogous upfield shift was also shown by the equivalent anomeric proton signals at δ_H 5.04 (2H, d, J = 9 Hz) of the symmetrical dimer isohirtellin C glucose cores (**13**), substantiating this.

In view of the above data, it is evident that macrocyclic dimers are formally produced due to bridging of a DHDG and an isoDHDG or two of either one of these moieties between O-1 and O-2 of the two glucose cores. In the hellinoyl tannins, two galloyl parts of a hellinoyl moiety are linked to O-1 and O-2 of a glucose core, forming a rigid 12-membered ring, whereas the remaining galloyl part either is connected to O-2 of another glucose core, leading to dimeric structures, or remains free, leading to the monomers.

Cytotoxic Activity of Tannins. Early studies have shown that several oligomeric ellagitannins exhibit in vivo antitumor activity against sarcoma 180 and MM2 in mice, which was attributed to an enhanced host immune response.[21−23] In another study, oligomeric ellagitannins exhibited in vivo antitumor (against S-180 in mice) and in vitro cytotoxic (against cancer cell lines) activities; thus, direct cytotoxicity and host-mediated antitumor mechanisms were suggested.[24] Recently, in vitro studies conducted with tumor cell lines have shown that several monomeric, dimeric, and oligomeric ellagitannins and their building units, the gallic and ellagic acids, exhibit potent cytotoxicity against carcinoma cell lines and lower cytotoxicity to normal cells.[15,25−27] In the present study, major *T. nilotica* dimers (**1**−**3**), together with some related tannins (**5**−**7** and **9**) with good abundance in the plant, were tested for their possible direct cytotoxic activity on human oral squamous cell carcinoma (HSC-2, HSC-3, and HSC-4) and promyelocytic leukemia (HL-60) cell lines compared with their effect on human oral normal cells (HGF, HPC, and HPLF). The results, presented in Table 5, clearly showed that all of the tested tannins were potently cytotoxic, as indicated by the CC_{50} and high tumor specificity (TS) values against HL-60 cells. The results also revealed that the DHDG possessing monomeric (**5** and **6**) and linear dimeric (**1**) tannins exhibited relatively higher cytotoxicity against HL-60 cells than did the hellinoyl tannins (**2**, **3**, **7**, and **9**). Except for remurin B (**6**), all of the tested compounds generally showed noticeable cytotoxicity against HSC-2, HSC-3, and HSC-4 cells. Among all of the tested tannins, hirtellin A (**1**) and nilotinin D8 (**9**) showed potent cytotoxic effects and elevated TS values at the applied concentrations against all tested tumor cell lines. These results suggested that the cytotoxic effects of tannins are largely dependent on the structure and that they do not simply increase with molecular weight. Tannins isolated from *T. nilotica* could be candidates for developing low-toxicity antitumor agents.

Extracts of *Tamarix* plants have been used in traditional Egyptian medicine, mainly as an antiseptic agent and for dyeing and tanning purposes.[28] We reported here the isolation and characterization of 12 tannins including four new ones from the extract of *T. nilotica*. New findings concerning the cytotoxic activities of some of the *T. nilotica* tannins are also shown.

Experimental Section

General Experimental Procedures. The instruments for measurements of the optical rotations, UV spectra, CD spectra, NMR spectra, and mass spectra used in this study were the same as those cited in the previous reports.[1,14] Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC, Kyoto, Japan) column (4.6 i.d. × 250 mm) developed with *n*-hexane/MeOH/THF/HCO_2H (47:39:13:1) containing oxalic acid (450 mg/L) (flow rate, 1.5 mL/min; 280 nm UV detection) at room temperature. Analytical reversed-phase HPLC was performed on a YMC-Pack ODS-A A-303 column (4.6 i.d. × 250 mm) eluted with 0.01 M H_3PO_4/0.01 M KH_2PO_4/MeOH (2:2:1) (flow rate, 1 mL/min; 280 nm UV detection) at 40 °C. Preparative reversed-phase HPLC was performed at 40 °C on a YMC-Pack ODS-A A-324 column (10 i.d. × 300 mm) using 0.01 M H_3PO_4/0.01 M KH_2PO_4/MeOH [either 2:2:1 (solvent I) or 41.5:41.5:17 (solvent II)], at a flow rate of 2 mL/min with detection at 280 nm UV. The tumor cell lines were obtained from Riken Bioresource Center, Tsukuba, Ibaraki, Japan. The normal cells were prepared from periodontal tissues, according to the guidelines of the institutional board of Meikai University Ethics Committee (no. 0707) after obtaining informed consent from the patients. Because HGF, HPC, and HPLF cells have a limited lifespan due to in vitro senescence, these cells were used at a population doubling level of 5−8.

Plant Material. Leaves of *T. nilotica* were collected at Al-Wadi Al-Assiuty, 20 km northeast of Assiut city, Egypt, in October 2006, and identified by Prof. Mo'men Mostafa Zareh, Department of Botany, Faculty of Science, Assiut University. A voucher specimen (No. 1024) is deposited in the same department.

Extraction and Isolation. The 40% aqueous MeOH fraction (10.4 g), which was obtained from the chromatographic fractionation of a 70% aqueous acetone homogenate of *T. nilotica* dried leaves (200 g) on a Diaion HP-20 column, was chromatographed over a Toyopearl HW-40 (coarse) column as described previously.[1,14] The Toyopearl fractions (Tfrs) 20−23, eluted with 70% EtOH(aq), afforded crude

tannin (19 mg), which was purified by preparative HPLC with solvent I as an eluent, to give 1,2,6-tri-*O*-galloyl-*β*-D-glucose[17] (**14**) (2 mg). The Tfrs 40−43, eluted with 70% EtOH(aq), afforded tellimagrandin I[16] (26 mg). The Tfrs 70−97 (240 mg), eluted with 70% aqueous EtOH, were subjected to an MCI gel CHP-20P column (1.1 i.d. × 22 cm) and eluted with aqueous MeOH (10% → 20% → 25% → 30% → 40%) and 100% MeOH. Nilotinin D8 (**9**) (8 mg) and tellimagrandin II[16] (4 mg) were purified from the 25% MeOH(aq) eluate (26 mg) and the 30% MeOH(aq) eluate (29 mg), respectively, by preparative HPLC with solvent I. The Tfrs 98−190 (1.7 g), eluted with 70% EtOH(aq)/70% acetone(aq) (9:1, v/v), were further chromatographed over a Sephadex LH-20 column (2.2 i.d. × 32 cm) with 70% EtOH(aq), collecting 700-drop fractions, and yielded Sephadex fractions (Sfr) i−iii. The Sfr-i (216 mg) was rechromatographed over the same Sephadex column with 70% EtOH(aq) and afforded six subfractions (Sub) A−F. The Sub B (18 mg) fraction was subjected to an MCI-gel CHP-20P (1.1 i.d. × 21 cm) column with 25% MeOH(aq) and yielded pure nilotinin M4 (**7**) (2 mg). A preparative Sub C (13 mg) HPLC purification with solvent II furnished an additional pure sample of nilotinin M4 (**7**) (4 mg). Sub D (27 mg) showed a mixture of dimeric tannins with the same retention time (t_R 7.78 min) on NP-HPLC. HPLC purification of the mixture with solvent II yielded the isomeric tannins nilotinin D9 (**10**) (3 mg) and hirtellin F[9] (**12**) (6 mg). Sub E (19 mg) was subjected to an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) with H_2O, MeOH(aq) gradients (10% → 20% → 30% → 40%), and 100% MeOH, and the 40% MeOH(aq) eluate yielded nilotinin D7 (**8**) (4 mg). Sfr-ii (300 mg) was also rechromatographed on the same Sephadex column with 70% EtOH(aq) in the isocratic mode, and the late eluted fractions were combined (88 mg) and subjected to an MCI-gel CHP-20P column (1.1 i.d. × 35 cm) with H_2O, MeOH(aq) (10% → 20% → 25% → 30% → 40%), and 100% MeOH. The eluate with 20% MeOH(aq) afforded tamarixinin A[8] (**3**) (20 mg) and isohirtellin C[9] (**13**) (5 mg) in the subsequent fractions, and the eluate with 25% MeOH(aq) gave hirtellin B[7] (**2**) (30 mg). Sfr-iii (644 mg) was further fractionated on an MCI-gel CHP-20P column (1.1 i.d. × 35 cm) with H_2O, MeOH(aq) gradients (10% → 20% → 25% → 30% → 40%), and 100% MeOH. The early eluate with 20% MeOH(aq) gave the main part of tamarixinin A (**3**) (76 mg), whereas the late eluate (45 mg) was further submitted to an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) with 20% MeOH(aq) to give crude tannin (19 mg). This afforded pure isohirtellin C (**13**) (11 mg) after HPLC purification with solvent I. The eluate with 25% MeOH(aq) (296 mg) was rechromatographed on the same MCI-gel column with 20% MeOH(aq) to give an additional pure sample of hirtellin B (**2**) (123 mg) and an impure fraction (117 mg). The latter fraction was purified by HPLC with solvent I to give an additional pure sample of **2** (51 mg). Tfrs 191−218 (285 mg), eluted with 70% EtOH(aq)/70% acetone(aq) (9:1, v/v), were submitted to an MCI-gel CHP-20P column (1.1 i.d. × 35 cm) with H_2O, MeOH(aq) gradients (10% → 20% → 30% → 35%), and 100% MeOH. A crude tannin (30 mg) in the 35% MeOH(aq) eluate was further purified by HPLC with solvent I to afford pure hirtellin C[9] (**11**) (21 mg) and an additional isohirtellin C fraction (**13**) (3 mg).

In a separate experiment, another quantity (1 kg) of the same dried leaves was extracted and fractionated according to the procedures followed in the first experiment. Subsequently, enrichments of **7** (14 mg), **8** (6 mg), **9** (11 mg), **10** (14 mg), and **12** (15 mg) from corresponding fractions were achieved.

Nilotinin M4 (7): off-white, amorphous powder; $[\alpha]^{29}_D$ +107.9 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 220 (5.15), 275 (4.79); CD (MeOH) [θ] (nm) +2.0 × 10^5 (238), −3.9 × 10^4 (262), +3.1 × 10^4 (283); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.64 (1H, s, hellinoyl H-6″), 7.09 (1H, d, J = 2.4 Hz, hellinoyl H-6), 6.89 (2H, s, galloyl H-2/H-6), 6.74 (1H, s, hellinoyl H-6′), 6.60 (1H, s, HHDP H-3), 6.46 (1H, s, HHDP H-3′), 6.10 (1H, d, J = 2.4 Hz, hellinoyl H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 3; ESIMS *m/z* 1127 [M + Na]$^+$, *m/z* 1122 [M + NH_4]$^+$, and *m/z* 1105 [M + H]$^+$; *anal.* C 43.63%, H 4.24%, calcd for $C_{48}H_{32}O_{31}\cdot 12H_2O$, C 43.59%, H 3.85%.

Nilotinin D7 (8): off-white, amorphous powder; $[\alpha]^{25}_D$ +84.9 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218 (5.47), 275 (5.14); CD (MeOH) [θ] (nm) +3.4 × 10^5 (232), −5.6 × 10^4 (263), +4.2 × 10^4 (287); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.56 (1H, s, hellinoyl H-6″), 7.12 [2H, s, galloyl-C (H-2/H-6)], 7.03 (2H, s, galloyl-D (H-2/H-6)], 6.94, [2H, s, galloyl-A (H-2/H-6)], 6.93 [2H, s, galloyl-B (H-2/H-6)], 6.79 (1H, d, J = 2.4 Hz, hellinoyl H-6), 6.71 (1H, s, HHDP H-3), 6.64 (1H, s, hellinoyl H-6′), 6.50 (1H, s, HHDP H-3′), 5.80 (1H, d, J = 2.4 Hz, hellinoyl H-2), and glucoses protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 3; ESIMS *m/z* 1745 [M + Na]$^+$; HRESIMS *m/z* 1745.16872 [M + Na]$^+$ (calcd for $C_{75}H_{54}O_{48}Na$, 1745.16767).

Nilotinin D8 (9): off-white, amorphous powder; $[\alpha]^{21}_D$ +128.8 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218 (5.29), 278 (4.95); CD (MeOH) [θ] (nm) +3.4 × 10^5 (233), −4.2 × 10^4 (262), +3.4 × 10^4 (287); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.56 (1H, s, hellinoyl H-6″), 7.00 [2H, s, galloyl-C (H-2/H-6)], 6.98 [2H, s, galloyl-A (H-2/H-6)], 6.90 [2H, s, galloyl-B (H-2/H-6)], 6.78 (1H, d, J = 1.8 Hz, hellinoyl H-6), 6.68 (1H, s, hellinoyl H-6′), 6.60 (1H, s, HHDP H-3), 6.55 (1H, s, HHDP H-3′), 5.68 (1H, d, J = 1.8 Hz, hellinoyl H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 3; ESIMS *m/z* 1593 [M + Na]$^+$; *anal.* C 47.11%, H 3.92%, calcd for $C_{68}H_{50}O_{44}\cdot 9H_2O$, C 47.05%, H 3.72%.

Hirtellin B (2): off-white, amorphous powder; ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.56 (1H, s, hellinoyl H-6″), 6.99 [2H, s, galloyl-A (H-2/H-6)], 6.90 [2H, s, galloyl-C (H-2/H-6)], 6.89 [2H, s, galloyl-B (H-2/H-6)], 6.81 (1H, d, J = 2.4 Hz, hellinoyl H-6), 6.70, 6.60 (each 1H, s, HHDP H-3 × 2), 6.66 (1H, s, hellinoyl H-6′), 6.55, 6.47 (each 1H, s, HHDP H-3′ × 2), 5.72 (1H, d, J = 2.4 Hz, hellinoyl H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Table 3; ESIMS *m/z* 1895 [M + Na]$^+$.

Tamarixinin A (3): off-white, amorphous powder; ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) (α- and *β*-anomers) δ_H 7.63, 7.60 (each s, 1H in total, hellinoyl H-6″), 7.05, 7.03 (each d, J = 2.4 Hz, 1H in total, hellinoyl H-6), 6.94, 6.93 (each s, 2H in total, galloyl-A (H-2/H-6)], 6.892, 6.886 [each s, 2H in total, galloyl-B (H-2/H-6)], 6.72, 6.71 (each s, 1H in total, hellinoyl H-6′), 6.625, 6.624, 6.615, 6.605 [each s, 2H in total, HHDP H-3′ × 2], 6.52, 6.51, 6.468, 6.465 [each s, 2H in total, HHDP H-3 × 2], 6.02 (1H, d, J = 2.4 Hz, hellinoyl H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 3; ESIMS *m/z* 1743 [M + Na]$^+$.

Nilotinin D9 (10): off-white, amorphous powder; $[\alpha]^{22}_D$ +42.5 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218.5 (5.35), 276.5 (5.02); CD (MeOH) [θ] (nm) +1.7 × 10^5 (231), −5.3 × 10^4 (266), +2.6 × 10^4 (288); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.11 (1H, s, DHDG H-6′), 7.10 (1H, d, J = 1.8 Hz, DHDG H-6), 7.05 [2H, s, galloyl-A (H-2/H-6)], 6.97 [2H, s, galloyl-B (H-2/H-6)], 6.84 [2H, br s, isoDHDG (H-2/H-6)], 6.78 (1H, br s, isoDHDG H-6′), 6.58 (1H, s, HHDP H-3), 6.48 (1H, s, HHDP H-3′), 6.44 (1H, br d, J = 1.8 Hz, DHDG H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 4; ESIMS *m/z* 1593 [M + Na]$^+$; HRESIMS *m/z* 1593.15514 [M + Na]$^+$ (calcd for $C_{68}H_{50}O_{44}Na$, 1593.15673).

Hirtellin C (11): off-white, amorphous powder; $[\alpha]^{20}_D$ +44.9 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 219 (5.52), 276 (5.20); CD (MeOH) [θ] (nm) +2.6 × 10^5 (236), −7.2 × 10^4 (266), +3.5 × 10^4 (288); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.15 (1H, s, DHDG H-6′), 7.10 (1H, d, J = 1.8 Hz, DHDG H-6), 6.96, 6.95 [each 2H, s, galloyl (H-2/H-6) × 2], 6.89 [2H, br s, isoDHDG (H-2/H-6)], 6.81 (1H, br s, isoDHDG H-6′), 6.582, 6.579 (each 1H, s, HHDP H-3 × 2), 6.481, 6.475 (each 1H, s, HHDP H-3′ × 2), 6.43 (1H, d, J = 1.8 Hz, DHDG H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 4; ESIMS *m/z* 1890 [M + NH_4]$^+$.

Hirtellin F (12): off-white, amorphous powder; $[\alpha]^{23}_D$ +28.5 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 219 (5.49), 276.5 (5.16); CD (MeOH) [θ] (nm) +1.7 × 10^5 (231), −6.2 × 10^4 (265), +3.5 × 10^4 (291); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.12 (1H, s, DHDG H-6′), 7.04 [2H, s, galloyl-B H-2/H-6)], 6.98 (1H, d, J = 1.8 Hz, DHDG H-6), 6.94 [2H, s, galloyl-A (H-2/H-6)], 6.87 [2H, br s, isoDHDG (H-2/H-6)], 6.83 (1H, br s, isoDHDG H-6′), 6.58 (1H, s, HHDP H-3), 6.48 (1H, s, HHDP H-3′), 6.41 (1H, d, J = 1.8 Hz, DHDG H-2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 4; ESIMS *m/z* 1593 [M + Na]$^+$; HRESIMS *m/z* 1593.15575 [M + Na]$^+$ (calcd for $C_{68}H_{50}O_{44}Na$, 1593.15672).

Isohirtellin C (13): off-white, amorphous powder; $[\alpha]^{26}_D$ +120.1 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 219.5 (2.48), 274 (5.18); CD (MeOH) [θ] (nm) +2.1 × 10^5 (227), +2.2 × 10^5 (237), −1.1 × 10^4 (258), +8.2 × 10^4 (277), +6.8 × 10^4 (310); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.17 (2H, s, DHDG H-6′ × 2), 7.08 (2H, d, J = 1.8 Hz, DHDG H-6 × 2), 6.90 [4H, s, galloyl (H-2/H-6) × 2], 6.57 (2H, s, HHDP H-3 × 2), 6.48 (2H, s, HHDP H-3′ × 2), 6.07 (2H, d,

$J = 1.8$ Hz, DHDG H-2 × 2), and glucose protons (Table 1); ^{13}C NMR spectroscopic assignments, see Tables 2 and 4; ESIMS *m/z* 1895 [M + Na]$^+$.

Cytotoxic Activity Assay. The cells (other than HL-60) were inoculated at 5×10^3 cells/well in 96-microwell plates (Becton Dickinson, Franklin Lakes, NJ) unless otherwise stated. After 48 h, the medium was removed by suction with an aspirator and replaced with 0.1 mL of fresh medium containing different test compound concentrations. Each test compound was dissolved in DMSO at a concentration of 80 mM. The first well contained 800 μM of the test compound and was sequentially diluted 2-fold, with three replicate wells for each concentration. The cells were incubated for an additional 48 h, and the relative viable cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, the cells were washed with phosphate-buffered saline without calcium and magnesium, which was replaced with fresh culture medium containing 0.2 mg/mL MTT, and the cells were incubated for another 4 h. The cells were lysed with 0.1 mL of DMSO, and the absorbance of the cell lysate at 540 nm (A_{540}) was determined using a microplate reader (Biochromatic Labsystem, Helsinki, Finland) (13). The A_{540} of the control cells was usually in the range from 0.40 to 0.90. The HL-60 cells were inoculated at 7.5×10^4 cells/0.1 mL in 96-microwell plates, and different concentrations of test compounds were added. After a 48 h incubation, the viable cell number was determined with a hemocytometer under a light microscope after trypan blue staining. The CC_{50} was determined from the dose−response curve.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for **7**−**12**, HSQC and HMBC spectra for **11**, and structures of the known tannins tellimagrandins I and II are available free of charge via the Internet at http://pubs.acs.org.

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