Dichapetalin-Type Triterpenoids and Lignans from the Aerial Parts of *Phyllanthus acutissima*

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Chemical investigation of the aerial parts of *Phyllanthus acutissima* resulted in the isolation of five new dichapetalin-type triterpenoids, acutissimatriterpenes A−E (**1**−**5**), and two new lignans, acutissimalignans A (**6**) and B (**7**), along with two known lignans and three known ellagic acid derivatives. The structures of **1**−**7** were determined mainly on the basis of spectroscopic methods. The compounds obtained were evaluated for cytotoxic and anti-HIV-1 activities.

Several species of *Phyllanthus* (Euphorbiaceae) have been used in traditional medicine.[1] Earlier investigations on plants in the genus *Phyllanthus* revealed the presence of flavonoids,[2] alkaloids,[3] terpenoids,[4] lignans,[5] and tannins.[6] We have reported recently a number of cytotoxic arylnaphthalide lignan glycosides from the aerial parts of *Phyllanthus taxodiifolius.*[7] As a continuation of our ongoing search for anticancer agents from plants, the chromatographic separation of hexane, ethyl acetate, and methanol extracts of the aerial parts of *P. acutissima* Miq. ("Phak wan chang khlong" or "Chan tia" in Thai) led to the isolation of five new phenylpyranotriterpenoids, acutissimatriterpenes A−E (**1**–**5**), two new lignans, 4-*O*-(2-*O*-methyl-δ-L-arabinopyranosyl)diphyllin (acutissimalignan A) (**6**) and (2*E*,3*S*)-2-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxy-3-methoxybenzyl)butyrolactone (acutissimalignan B) (**7**), and five known compounds, taiwanin C (**8**),[8] isogadian (**9**),[9] 3,3′,4′-tri-*O*-methylellagic acid (**10**),[10] 3′-mono-*O*-methylellagic acid 4-*O*-α-L-rhamnopyranoside (**11**),[11] and 3,3′,4′-tri-*O*-methylellagic acid 4-*O*-β-D-glucopyranoside (**12**).[12] Compounds **1**, **2**, **5**, and **9** were isolated from the hexane extract, while the ethyl acetate extract provided compounds **1**–**5** and **7**–**10**. Purification of the methanol extract yielded compounds **6**, **7**, **9**, **11**, and **12**. The isolation, characterization, and evaluation of these compounds for cytotoxic activities against a panel of six mammalian cancer cell lines, and anti-HIV-1 activities using both cell-based and RT assays, are described herein.

Results and Discussion

(+)-Acutissimatriterpene A (**1**) was determined to possess a molecular formula of $C_{40}H_{50}O_8$ from the $[M + Na]^+$ peak at *m*/*z* 681.3403 in the HRTOFMS. The fragment ion at *m*/*z* 640 $[M - H_2O]^+$ in the EIMS suggested that **1** is an alcohol. Compound **1** showed UV absorptions at 231 and 282 nm, while the IR spectrum indicated the presence of hydroxy (3583 cm^{-1}), a carbonyl of a five-membered lactone (1765 cm^{-1}), aliphatic C=C (1657 cm^{-1}), aromatic C=C (1610, 1505, and 1491 cm^{-1}), and methylenedioxy ether (936 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) displayed four tertiary methyl signals (δ 1.01, 1.08, 1.30, and 1.46), together with a pair of doublets of cyclopropyl methylene protons at δ 0.67 and 0.82 (J = 5.1 Hz each), indicating that **1** is a triterpene containing a cyclopropane ring. The HMBC correlations (Table S1, Supporting Information) of these cyclopropyl signals to C-8, C-12, C-13, C-14, C-15, and C-17 provided support for the location of the cyclopropylmethylene at C-30. Furthermore, the presence of a 3,4-methylenedioxyphenyl ring was indicated by the aromatic signals at δ 6.91, 6.82, and 6.77 (J_{ortho} = 8.0 Hz, J_{meta} = 1.6 Hz), together with a pair of doublets at δ 5.940 and 5.936 (J = 1.7 Hz) of the methylenedioxy protons. As HMBC correlations from C-6′ to H-2′a, H-2′b, H-2″, and H-6″ were observed, the 3,4-methylenedioxyphenyl ring was confirmed to be at the C-6′ position. Other ^1H NMR signals were assigned to the two olefinic methines (δ 5.36 and 6.83), two oxymethines (δ 3.84 and 4.16), two oxymethylenes at (δ 3.74/3.55 and 4.18/3.90), an aliphatic methoxy (δ 3.26), and a hydroxy (δ 2.41), including the signals arising from other methines and methylenes in a 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton with a spiro-lactone side chain. The HMBC correlations of C-20 to H-16b, H-17, and H-22; C-21 to H-17 and H-22; and C-22 to H-17, H-24a, and H-24b confirmed the attachment of the side chain to C-17. The five-membered spiro-lactone structure and the location of methyl and methoxyl groups at C-25 in the side chain were proved by the correlations of the spiro-carbon C-23 to H-22, H-24b, and H-26a; C-24 to H-26a and H-27; C-25 to H-24a, H-24b, H-26a, H-26b, H-27, and OCH_3-25; C-26 to H-24a, H-24b, and H-27; and C-27 to H-24a, H-24b, and H-26b. The ^{13}C NMR spectrum of **1** (Table 2) exhibited five methyls, 12 methylenes, 10 methines, 12 quaternary carbons, and one carbonyl carbon, of which the assignments were performed by 2D-NMR studies (COSY, HMQC, HMBC). The relative configuration of **1** was obtained from a NOESY experiment, as summarized in Figure 1, while single-crystal X-ray diffraction analysis of the *p*-bromobenzoate **1a** confirmed the absolute stereochemisty of its precursor **1** to be 4*R*, 5*R*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*S*, 17*S*, 23*S*, 25*R*, and 6′*S*. The ORTEP diagram of **1a** is shown in Figure 2. Triterpenes with a 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton similar to **1** but with a different side chain have been reported previously from *Dichapetalum madagascariense*[13a−c] and *D. gelonioides* (Dichapetalaceae).[13d]

The molecular formula ($C_{39}H_{50}O_6$) of acutissimatriterpene B (**2**) was established on the basis of HRTOFMS at *m*/*z* 637.3508 $[M + Na]^+$. The alcoholic character of this compound was suggested by the fragment ion at *m*/*z* 596 $[M - H_2O]^+$ in the EIMS. Compound **2** exhibited a UV absorption at 250 nm, indicating the presence of a different chromophore when compared to **1**. In general, the IR absorptions of **2** were found to be in accordance with those of **1**, except that the C−O absorption of a methylenedioxyphenyl group was not observed. Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** (Tables 1 and 2) showed close similarities, with the exception that the 3,4-methylenedioxyphenyl group at C-6′ in **1** was replaced by a phenyl group [δ 7.32–7.40 (4H, overlapping signals, H-2″, H-3″, H-5″, and H-6″) and 7.28 (m, H-4″)]. Therefore, a 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton with a phenyl substituent at C-6′ was proposed for **2**. By detailed analysis of the COSY, HMQC, and HMBC data (for the HMBC

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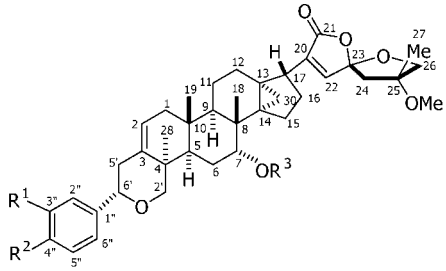
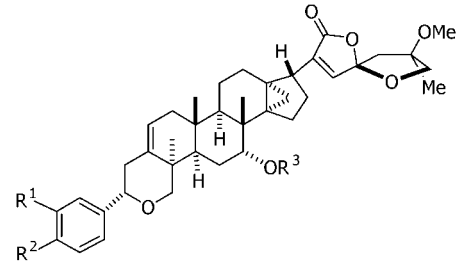
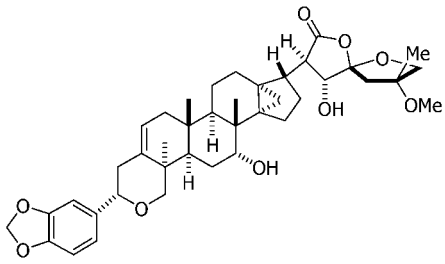


1 R^1, R^2 = O-CH_2-O, R^3 = H
1a R^1, R^2 = O-CH_2-O, R^3 = *p*-BrPhCO
2 R^1 = R^2 = R^3 = H



3 R^1, R^2 = O-CH_2-O, R^3 = H
3a R^1, R^2 = O-CH_2-O, R^3 = *p*-BrPhCO
4 R^1 = R^2 = R^3 = H



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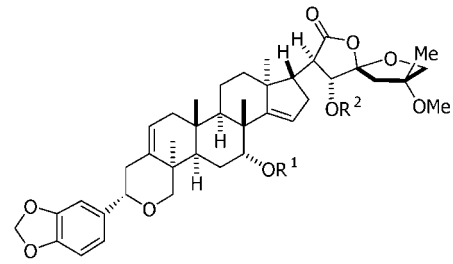
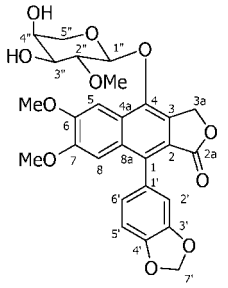


5a R^1 = R^2 = H
5b R^1 = H, R^2 = *p*-BrPhCO
5c R^1 = R^2 = *p*-BrPhCO



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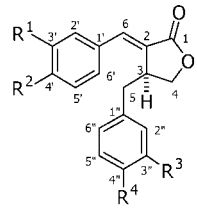
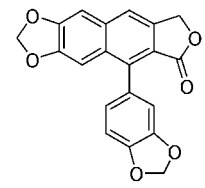


7 R^1 = R^3 = OMe, R^2 = R^4 = OH
9 R^1-R^2 = R^3-R^4 = O-CH_2-O



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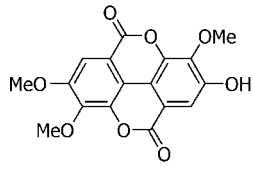

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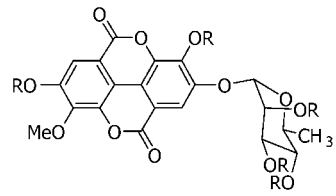
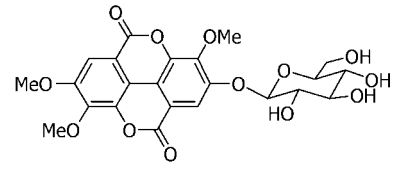


11 R = H
11a R = COMe



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values, see Table S1, Supporting Information), this proposal was confirmed. The relative configuration of **2** was determined to be identical to that of **1**, as judged from the NOESY spectrum. Since both compounds **1** and **2** gave positive optical rotation values and Cotton effects in their CD spectra, the absolute configuration of **2** was therefore concluded to be 4*R*, 5*R*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*S*, 17*S*, 23*S*, 25*R*, and 6′*S*, as in **1**.

The $[M + Na]^+$ peak at *m*/*z* 681.3403 in the HRTOFMS of acutissimatriterpene C (**3**) indicated that this compound has the same molecular formula ($C_{40}H_{50}O_8$) as **1**. In the EIMS of **3**, the fragment ion at *m*/*z* 640 $[M - H_2O]^+$ supported the presence of a hydroxyl group in the structure. Furthermore, both compounds exhibited closely comparable UV and IR data (see Experimental Section). Similar to **1**, the C−O absorption band at 935 cm^{-1} in the IR spectrum of **3** suggested the presence of at least one 3,4-methylenedioxyphenyl group in the structure. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were very similar to those of **1** and supported the basic 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton with a 3,4-methylenedioxyphenyl group at position C-6′ of **3**. However, slight shifts of the ^1H and ^{13}C NMR signals (H-22, H-24, H-26, H-27, and OCH_3-25; and C-22→C-27) in the side chain were observed. Full assignments of the ^1H and ^{13}C NMR signals, the connectivities, and the relative configuration of **3** were concluded from the detailed analysis of DEPT, COSY, HMQC, and HMBC spectra (for HMBC data, see Table S1, Supporting Information). The NOESY correlations of **3** (Figure 1) were generally similar to those of **1**. The only change was that H-22 exhibited a correlation to H-24b instead of H-24a, suggesting that **3** possesses the opposite configuration (23*R*) at C-23 when compared to **1**. Due to the co-occurrence of these two compounds in the same species of plant, the other chiral stereochemistries were assumed to be the same as in **1**. The difference of configuration at C-23 might be the result of

Table 1. ^{1}H NMR Spectroscopic Data of Triterpenoids **1–5**

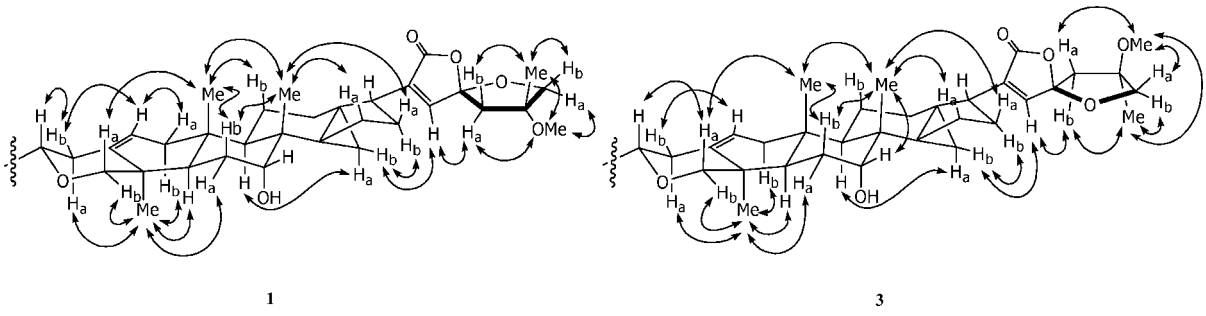
	1	**2**	**3**	**4**	**5**
position	δ_H mult. (J)[a]	δ_H mult. (J)[a]	δ_H mult. (J)[a]	δ_H mult. (J)[a]	δ_H mult. (J)[a]
1a	1.96 dd (16.2, 7.0)	1.97 dd (16.1, 6.9)	1.96 dd (16.7, 7.0)	1.95 dd (16.7, 6.9)	1.96 dd (16.3, 6.9)
1b	1.63 br d (16.2)	1.64 br d (16.1)	1.61 br d (16.7)	1.62 d (16.7)	1.61 obsc.
2	5.36 br d (7.0)	5.38 br d (6.9)	5.36 br d (7.0)	5.37 br d (6.9)	5.36 br d (6.9)
5	1.98 obsc.	2.00 obsc.	1.99 obsc.	1.99 obsc.	1.99 obsc.
6a	1.77 obsc.	1.78 obsc.	1.79 obsc.	1.78 obsc.	1.77 br d (13.7)
6b	1.52 ddd (13.7, 13.7, 2.1)	1.53 ddd (13.7, 13.7, 2.0)	1.52 ddd (13.7, 13.7, 2.2)	1.54 ddd (13.7, 13.7, 2.1)	1.51 ddd (13.7, 13.7, 2.1)
7	3.84 br m	3.84 br m	3.84 obsc.	3.84 obsc.	3.81 br m
9	1.32 obsc.	1.31 obsc.	1.30 obsc.	1.31 obsc.	1.30 obsc.
11a	1.39 obsc.	1.40 obsc.	1.41 obsc.	1.40 obsc.	1.41 obsc.
11b	1.30 obsc.	1.29 obsc.	1.28 obsc.	1.30 obsc.	1.31 obsc.
12a	1.87 ddd (14.3, 8.5, 3.2)	1.89 ddd (14.3, 8.6, 2.9)	1.86 ddd (14.2, 8.9, 3.0)	1.86 ddd (14.2, 8.9, 3.1)	1.87 br t (7.4)
12b	1.79 obsc.	1.78 obsc.	1.77 obsc.	1.77 obsc.	
15a	2.03 obsc.	2.03 obsc.	2.03 obsc.	2.03 obsc.	1.97 obsc.
15b	1.67 dd (12.0, 8.1)	1.68 dd (11.9, 8.2)	1.68 dd (12.0, 8.1)	1.66 dd (12.0, 8.1)	1.64 obsc.
16a	2.05 obsc.	2.07 obsc.	2.06 obsc.	2.07 obsc.	1.62 obsc.
16b	1.04 obsc.	1.04 obsc.	1.06 obsc.	1.07 obsc.	1.04 obsc.
17	2.86 br dd (10.5, 7.1)	2.87 br dd (10.5, 7.2)	2.86 br dd (10.6, 7.3)	2.87 br dd (10.5, 7.4)	2.97 dd (10.0, 4.4)
18	1.08 s	1.09 s	1.09 s	1.09 s	1.07 s
19	1.01 s	1.03 s	1.02 s	1.03 s	1.01 s
20					2.54 obsc.
22	6.83 d (1.1)	6.84 d (1.1)	6.73 d (1.4)	6.73 d (1.4)	4.15 d (10.0)
24a	2.48 dd (14.1, 1.1)	2.48 d (14.4)	2.57 d (14.3)	2.57 d (14.3)	2.49 dd (14.4, 1.0)
24b	2.26 d (14.1)	2.27 d (14.4)	2.11 d (14.3)	2.11 d (14.3)	2.30 d (14.4)
26a	4.18 dd (9.7, 1.0)	4.18 dd (9.6, 0.9)	4.26 d (9.1)	4.26 d (9.2)	4.13 dd (9.7, 1.0)
26b	3.90 d (9.7)	3.90 d (9.6)	3.85 d (9.1)	3.85 d (9.2)	3.86 d (9.7)
27	1.46 s	1.46 s	1.44 s	1.44 s	1.42 s
28	1.30 s	1.33 s	1.31 s	1.33 s	1.30 s
30a	0.82 d (5.1)	0.82 d (4.9)	0.81 d (4.9)	0.81 d (5.1)	0.77 d (5.0)
30b	0.67 d (5.1)	0.68 d (4.9)	0.70 d (4.9)	0.70 d (5.1)	0.66 d (5.0)
2′a	3.74 d (10.7)	3.77 d (10.7)	3.74 d (10.7)	3.77 d (10.7)	3.74 d (10.7)
2′b	3.55 d (10.7)	3.59 d (10.7)	3.56 d (10.7)	3.59 d (10.7)	3.55 d (10.7)
5′a	2.59 br t (13.5)	2.63 br t (13.4)	2.59 br t (13.3)	2.63 br t (13.3)	2.59 obsc.
5′b	2.13 dd (13.5, 2.6)	2.19 dd (13.4, 2.5)	2.14 dd (13.3, 2.6)	2.19 dd (13.3, 2.5)	2.13 dd (13.4, 2.5)
6′	4.16 dd (12.6, 2.6)	4.26 dd (12.5, 2.5)	4.17 dd (12.5, 2.6)	4.28 obsc.	4.17 dd (11.5, 2.5)
2″	6.91 d (1.6)	7.32–7.40 obsc.	6.91 d (1.6)	7.32–7.40 obsc.	6.90 d (1.5)
3″		7.32–7.40 obsc.		7.32–7.40 obsc.	
4″		7.28 m		7.28 m	
5″	6.77 d (8.0)	7.32–7.40 obsc.	6.77 d (8.0)	7.32–7.40 obsc.	6.76 d (8.0)
6″	6.82 dd (8.0, 1.6)	7.32–7.40 obsc.	6.82 dd (8.0, 1.6)	7.32–7.40 obsc.	6.82 dd (8.0, 1.5)
7″a	5.940 d (1.7)		5.940 d (1.6)		5.93 s
7″b	5.936 d (1.7)		5.936 d (1.6)		
OCH_3-25	3.26 s	3.26 s	3.31 s	3.31 s	3.26 s
OH-7	2.41 br s	2.43 br s	2.38 br s	2.42 br s	-[b]
OH-22					3.04 br s

[a] Spectra recorded at 500 MHz in $CDCl_3$, using TMS as an internal reference; J values (in Hz) in parentheses; obsc. = obscured signals. [b] Not observed.

Table 2. 125 MHz ^{13}C NMR Spectroscopic Data of Triterpenoids **1**–**5**

	1		**2**		**3**		**4**		**5**	
carbon	δ_C[a]		δ_C[a]		δ_C[a]		δ_C[a]		δ_C[a]	
1	40.1	CH_2	40.1	CH_2	40.2	CH_2	40.2	CH_2	40.1	CH_2
2	118.0	CH	118.0	CH	118.0	CH	117.9	CH	118.1	CH
3	139.5	C	139.5	C	139.6	C	139.8	C	139.8	C
4	38.3	C	38.3	C	38.3	C	38.4	C	38.2	C
5	43.7	CH	43.6	CH	43.7	CH	43.8	CH	43.6	CH
6	23.8	CH_2	23.8	CH_2	23.9	CH_2	23.9	CH_2	23.7	CH_2
7	73.9	CH	73.9	CH	73.9	CH	73.9	CH	73.8	CH
8	38.6	C	38.5	C	38.6	C	38.6	C	38.3	C
9	42.4	CH	42.4	CH	42.5	CH	42.5	CH	43.7	CH
10	36.7	C	36.6	C	36.7	C	36.7	C	36.6	C
11	16.3	CH_2	16.2	CH_2	16.3	CH_2	16.3	CH_2	16.2	CH_2
12	25.3	CH_2	25.2	CH_2	25.5	CH_2	25.5	CH_2	25.0	CH_2
13	27.9	C	27.9	C	27.8	C	27.9	C	26.4	C
14	35.3	C	35.3	C	35.4	C	35.5	C	34.3	C
15	26.8	CH_2	26.8	CH_2	26.9	CH_2	26.9	CH_2	26.0	CH_2
16	27.7	CH_2	27.7	CH_2	27.7	CH_2	27.8	CH_2	21.8	CH_2
17	43.0	CH	42.9	CH	43.1	CH	43.1	CH	46.5	CH
18	19.7	CH_3	19.7	CH_3	19.7	CH_3	19.7	CH_3	19.5	CH_3
19	16.7	CH_3	16.7	CH_3	16.7	CH_3	16.7	CH_3	16.6	CH_3
20	138.4	C	138.4	C	139.1	C	139.2	C	42.4	CH
21	171.1	C	171.1	C	170.9	C	170.9	C	174.7	C
22	143.2	CH	143.1	CH	142.5	CH	142.5	CH	72.0	CH
23	113.3	C	113.3	C	113.7	C	112.6	C	112.2	C
24	46.9	CH_2	46.8	CH_2	45.1	CH_2	45.1	CH_2	45.3	CH_2
25	83.4	C	83.3	C	82.3	C	82.3	C	82.5	C
26	77.2	CH_2	77.3	CH_2	79.3	CH_2	79.3	CH_2	76.8	CH_2
27	20.1	CH_3	20.1	CH_3	22.1	CH_3	22.2	CH_3	19.0	CH_3
28	23.8	CH_3	23.8	CH_3	23.8	CH_3	23.8	CH_3	23.7	CH_3
30	14.1	CH_2	14.1	CH_2	14.2	CH_2	14.2	CH_2	14.4	CH_2
2′	72.6	CH_2	72.6	CH_2	72.6	CH_2	72.6	CH_2	72.5	CH_2
5′	40.7	CH_2	40.7	CH_2	40.8	CH_2	40.8	CH_2	40.7	CH_2
6′	81.6	CH	81.7	CH	81.6	CH	81.8	CH	81.5	CH
1″	136.7	C	142.6	C	136.7	C	142.7	C	136.7	C
2″	106.6	CH	125.8	CH	106.6	CH	125.8	CH	106.5	CH
3″	147.6	C	128.3	CH	147.6	C	128.4	CH	147.5	C
4″	146.8	C	127.4	CH	146.8	C	127.5	CH	146.7	C
5″	108.0	CH	128.3	CH	108.0	CH	128.4	CH	108.0	CH
6″	119.2	CH	125.8	CH	119.2	CH	125.8	CH	119.1	CH
7″	100.9	CH_2			100.9	CH_2			100.8	CH_2
OCH_3-25	50.8	CH_3	50.8	CH_3	51.3	CH_3	51.3	CH_3	50.6	CH_3

[a] Spectra recorded at 125 MHz in $CDCl_3$, using the $CDCl_3$ signal at δ_C 77.0 as reference; attached protons determined by DEPT experiments.



1 **3**

Figure 1. Observed NOESY correlations of acutissimatriterpenes A (**1**) and C (**3**).

the alternative mode of cyclization. Since the absolute configuration of **1** was established by single-crystal X-ray diffraction analysis of the *p*-bromobenzoate derivative **1a** as mentioned earlier, the absolute stereochemistry of **3** was therefore assigned as 4*R*, 5*R*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*S*, 17*S*, 23*R*, 25*R*, and 6′*S*. It is assumed that the *R*-configuration at C-23 is responsible for the negative Cotton effect in the CD spectrum of **3**.

Acutissimatriterpene D (**4**) exhibited an $[M + H]^+$ peak at *m*/*z* 615.3669, corresponding to the molecular formula of $C_{39}H_{50}O_6$ in the HRTOFMS. Its IR and UV absorptions were similar to those of **2**, which suggested that **4** also possesses a 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton with a C-6′-phenyl substituent. When compared to **2**, the ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** were different from only the NMR signals of the side chain (H-22, H-24, H-26, H-27, and OC*H*$_3$-25; and C-22→C-27), but were found to be identical to those of **3**. Analysis of the COSY, HMQC, and HMBC spectra (for HMBC data see Table S1, Supporting Information) enabled the assignments of all ^1H and ^{13}C NMR signals, as well as the connectivities within the molecule. Moreover, as compound **4** showed a negative Cotton effect profile similar to that of **3**, the absolute configuration of **4** was established as 4*R*, 5*R*, 7*R*, 8*R*, 9*R*, 10*S*, 13*R*, 14*S*, 17*S*, 23*R*, 25*R*, and 6′*S*, as in **3**.

The HRTOFMS of compound **5** showed the $[M + H]^+$ peak at *m*/*z* 677.3690, indicating a molecular formula of $C_{40}H_{52}O_9$. The peaks for $[M - H_2O]^+$ and $[M - 2H_2O]^+$ at *m*/*z* 658 and 640, respectively, in the EIMS supported **5** as a diol. Its IR spectrum showed a band for the C=O stretch of a saturated γ-lactone at 1784 cm^{-1}, in addition to the absorbances corresponding to hydroxyl,

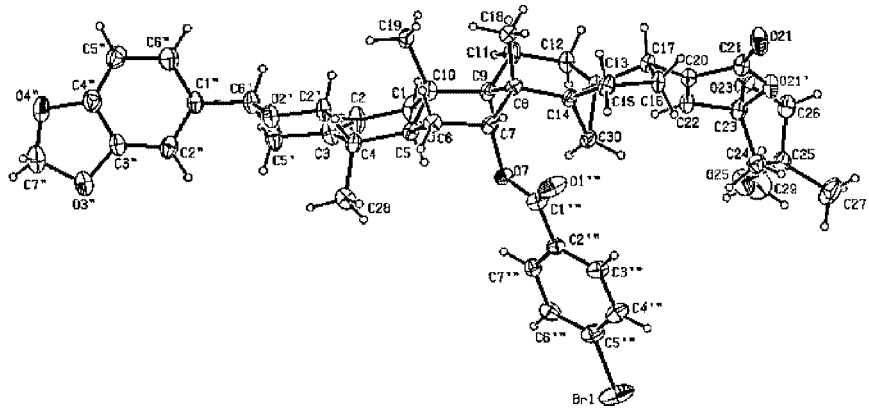


Figure 2. X-ray ORTEP diagram of compound **1a**.

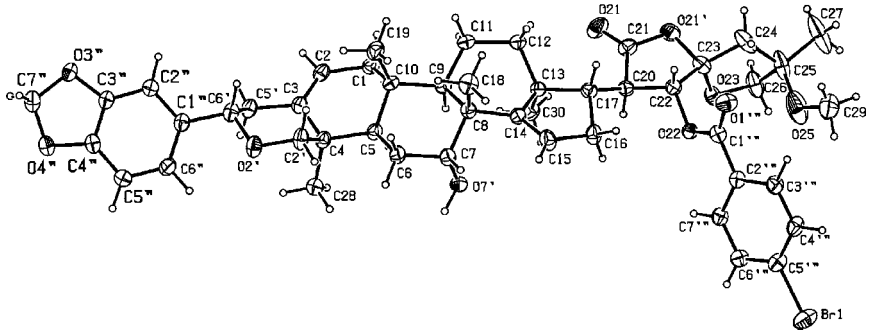
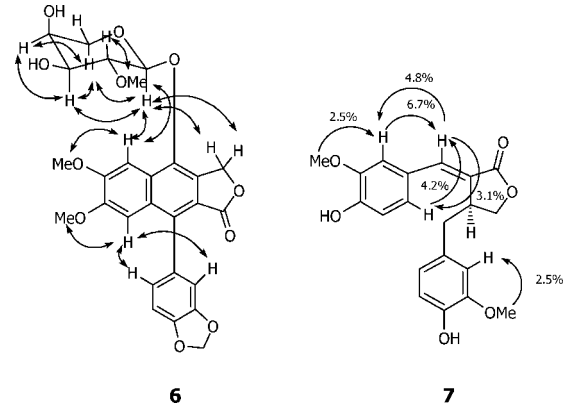


Figure 3. X-ray ORTEP diagram of *p*-bromobenzoate **5b**.

olefinic C═C, aromatic C═C, and methylenedioxy ether functionalities, which were consistent with those of **1**. Relevant portions of the ^{1}H NMR spectrum (Table 1) were similar to those of **1**. The major differences were the loss of one olefinic signal with an additional broad singlet (δ 3.04) corresponding to one more hydroxyl group, which was proposed to be located at the C-22 position by the observed HMBC correlation (Table S1, Supporting Information) between C-22 and OH-22. However, slight shifts of the proton and carbon signals around C-22 were also observed. Assignments of the ^{1}H and ^{13}C NMR signals were performed through analysis of COSY, HMQC, and HMBC correlation data. Thus, the structure of **5** was proposed as possessing a 13,30-cyclo-29-nordammarano[4,3-*c*]pyran skeleton with a 3,4-methylenedioxyphenyl group at position C-6′ and a saturated spiro-lactone side chain containing a 22-hydroxy group. When the NOESY spectrum of **5** was compared to that of **1**, the relative configurations were confirmed to be the same. Further proof for the absolute stereochemistry of **5** was carried out through the alkene **5a** because **5** was not stable. Under a trace of acid in $CDCl_3$ solution, cyclopropane ring opening occurred and led to the formation of **5a** during the recording of NMR data. Compound **5a** was further converted to the mono-*p*-bromobenzoate **5b** and di-*p*-bromobenzoate **5c** in 54 and 15% yields, respectively. By single-crystal X-ray diffraction analysis of **5b**, the absolute configurations of **5b** and its precursors **5a** and **5** were determined as 4*R*, 5*R*, 7*R*, 8*R*, 9*R*, 10*S*, 13*S*, 17*S*, 20*R*, 22*R*, 23*S*, 25*R*, and 6′*S*. The X-ray ORTEP diagram of **5b** is shown in Figure 3.

Acutissimalignan A (**6**) showed an $[M + H]^+$ peak at *m*/*z* 527.1542 in its HRTOFMS, establishing a molecular formula of $C_{27}H_{26}O_{11}$. The IR spectrum displayed the bands for hydroxy (3566 cm^{-1}), γ-lactone (1758 cm^{-1}), an aromatic (1625, 1600, and 1507 cm^{-1}), and methylenedioxy ether (936 cm^{-1}) functionalities. The ^{1}H NMR spectrum of **6** showed typical signals of an arylnaphthalide lignan monoglycoside with the doubling of some signals due to some degree of restricted rotation around the aryl−naphthalene bond[14] as indicated by an asterisk (*), in the Experimental Section.



6 **7**

Figure 4. Observed NOE correlations in lignan **6** and NOE enhancements in lignan **7**.

The two aromatic singlets at δ 7.80 and 7.10 (1H each, s each), together with the signals of a 1,3,4-trisubstituted benzene moiety at δ 6.96 (1H, d) and 6.79−6.85 (2H, overlapping signals), a lactone methylene at δ 5.571/5.565* and 5.459/5.455*, a methylenedioxy group at δ 6.10 and 6.054/6.052*, and two aromatic methoxyls at δ 4.07 and 3.82, suggested that **6** is a diphyllin analogue. The sugar moiety was identified as 2-*O*-methyl-α-L-arabinopyranose by analysis of the *J* values, as well as the ^{13}C NMR and 2D-NMR data (see Experimental Section). The location of the methoxyl group at the C-2″ position was confirmed by the HMBC correlation from C-2″ to the OC*H*$_3$-2″ (Table S2, Supporting Information), while the α-linkage between the sugar and the diphyllin moiety was deduced from a NOE correlation experiment (Figure 4). On the basis of this evidence, the structure of **6** was established as 4-*O*-(2-*O*-methyl-α-L-arabinopyranosyl)diphyllin.

Acutissimalignan (**7**) was determined to possess a molecular formula of $C_{20}H_{20}O_6$ from the $[M + H]^+$ peak at *m*/*z* 357.1313 in the HRTOFMS. It exhibited UV maxima at 229, 286, and 329 nm,

Table 3. Cytotoxic and Anti-HIV-1 Activities of the Isolated Compounds **1**–**11** and Modified **11a**

	cytotoxic activity[a] against cancer cell lines (μg/mL)						anti-HIV-1 activity[b]			
							cell-based assay (μg/mL)			RT assay
compound	P-388	KB	Col-2	MCF-7	Lu-1	ASK	IC_{50}	EC_{50}	SI	% inhibition at 200 μg/mL
1	0.5	>5	>5	>5	>5	>5	>125	86.1	>1.5	55.5
2	0.4	>5	>5	>5	>5	>5	>125	72.9	>1.7	65.9
3	>5	>5	>5	>5	>5	>5	>125	69.8	>1.8	11.0
4	>5	>5	>5	>5	>5	>5	12.9	5.1	2.5	37.8
5	0.005	>5	4.8	1.1	3.1	>5	31.6	<3.9	>8.1	−0.5
6	0.02	0.59	0.25	0.22	0.14	2.3	12.3	<3.9	>3.1	10.7
7	>5	>5	>5	>5	>5	>5	48.2	9.9	4.9	45.4
8	>5	>5	>5	>5	>5	>5	>125	>125		88.2
9	>5	>5	>5	>5	>5	>5	>125	34.6	>3.6	39.7
10	>5	>5	>5	>5	>5	>5	ND	ND		ND
11	>5	>5	>5	>5	>5	>5	>125	>125		−9.2
11a	>5	>5	>5	>5	>5	>5	24.9	15.7	1.6	51.0
ellipticine	0.2	0.7	0.5	0.4	0.2	0.2				

[a] $ED_{50} \leq 5$ μg/mL is considered active. P-388 = murine lymphocytic leukemia, KB = human nasopharyngeal carcinoma, Col-2 = human colon cancer, MCF-7 = human breast cancer, Lu-1 = human lung cancer, ASK = rat glioma. [b] Cell-based assay; EC_{50} > 125 μg/mL is considered insignificant; EC_{50} AZT, averaged from three experiments, 1.2×10^{-9} M; SI (selective index), IC_{50}/EC_{50}. RT assay; less than 30% inhibition of RT at 200 μg/mL is considered inactive; nevirapine (2 μg/mL), averaged from three experiments, 49.1% inhibition. ND, not determined.

characteristic of a dibenzylbutyrolactone lignan with a double bond at the C-2,C-6 positions.[15] The IR spectrum indicated the presence of hydroxyl (3539 cm^{-1}) and conjugated γ-lactone (1743 cm^{-1}) functionalities in **7**. The ^1H and ^{13}C NMR spectra of **7** (see Experimental Section) revealed signals for a dibenzylbutyrolactone lignan with a double bond at the C-2,C-6 positions, which were closely similar to those of (+)-7,8-didehydroarctigenin previously isolated from the fruits of *Arctium lappa*,[16] except for having only two methoxyl signals in the structure. As the H-2′ and H-2″ signals (δ 7.03 and 6.64) were both enhanced by 2.5% in NOE experiments, when the signals at δ 3.92 (OC*H*$_3$-3′) and 3.86 (OC*H*$_3$-3″) were irradiated, the locations of the two methoxyl groups were confirmed to be at C-3′ and 3″, respectively.

The *E*-configuration of the $\Delta^{2,6}$-double bond was indicated by the downfield olefinic signal of H-6 at δ 7.52, which was deshielded by the adjacent carbonyl group. By comparison of its optical rotation value {$[\alpha]^{25}_D$ +29.8 (*c* 0.63, $CHCl_3$)} with those of the previously known (+)-3*S*-lignan isolated from *Jatropha gossypifolia* {$[\alpha]^{25}_D$ +87 ($CHCl_3$)}[17] and (−)-3*R*-hibalactone {$[\alpha]^{22}_D$ −88 ($CHCl_3$); $[\alpha]^{23}_D$ −87 ($CHCl_3$)}[18a,b] isolated from *Juniperus sabina* and *Chamaecyparis obtusa*, the absolute configuration at C-3 of (+)-**7** was deduced as *S* and confirmed by the observed positive Cotton effect[18c] in the CD spectrum of (+)-**7**.

The structures of other known compounds (**8**–**12**) were identified by direct comparison of their physical and spectroscopic properties, which were in accordance with those reported in the literature. The lignans taiwanin C (**8**) and isogadian (**9**) have been previously reported from *Taiwania cryptomerioides*[8] and *Jatropha gossypifolia*,[9] respectively. The ellagic acid derivative 3,3′,4′-tri-*O*-methylellagic acid (**10**) has been obtained from *Diplopanax stachyanthus*,[10] while the glycosides 3′-mono-*O*-methylellagic acid 4-*O*-α-L-rhamnopyranoside (**11**) and 3,3′,4′-tri-*O*-methylellagic acid 4-*O*-β-D-glucopyranoside (**12**) have been previously isolated from *Eucalyptus globulus*[11] and *Eucalyptus polyanthemos*,[12] respectively.

The results of biological testing of the pure compounds **1**–**11** and **11a** are given in Table 3. When tested for cytotoxic effects against a panel of cancer cell lines,[19] triterpenes **1** and **2** exhibited such activity only against the P-388 cell line, whereas triterpene **5** showed significant activities for the P-388, MCF-7, and Lu-1 cell lines. Lignan **6** was found active in all cell lines tested. Anti-HIV-1 activities were also evaluated employing cell-based cytotoxic and syncytium assays using $^{\Delta Tat/Rev}$MC99 virus and 1A2 cell line system,[20] as well as HIV-1 reverse transcriptase (RT) assay.[21] The cell-based assay for anti-HIV-1 activity revealed that all new isolated compounds **1**−**7**, the known lignan **9**, and the modified ellagic acid glycoside **11a** were active, while lignan **8** and compound **11** were inactive. In the HIV-1 RT assay, the known lignan **8** was most active (88.2% inhibition at 200 μg/mL), while triterpenes **1** and **2** and the modified ellagic acid glycoside **11a** were moderately active (>50 to 70% inhibition). The scarcity of compound **10** made it unavailable for evaluation in the anti-HIV-1 assays.

The occurrence of dichapetalin-type triterpenes in nature is rare. A few of these compounds have been reported so far, but only from the genus *Dichapetalum*.[13] From the roots of *D. madagascariense*, dichapetalins A−H were isolated and structurally identified,[13a−c] and the absolute configuration of dichapetalin A was determined by using X-ray crystallography.[13c] Dichapetalin A was reported to exhibit strong activity in the brine shrimp lethality bioassay, exceeding that of podophyllotoxin by 7-fold, and also showed cytotoxicity against L1210 murine leukemia cells (EC_{90} < 0.0001 μg/mL), while the KB and GM-CSF stimulated murine bone marrow cell lines were less sensitive.[13a] Dichapetalins A, I, and J, isolated from the EtOAc-soluble extract of the stem bark of *D. gelonioides*, were reported to exhibit promising selectivity against the SW626 (human ovarian cancer) cell line, but the dichapetalins K and L isolated from the re-collected plant material showed broad cytotoxic activity when tested against a panel of human tumor cell lines.[13d] Dichapetalin A, isolated from the latter species, was found inactive when evaluated in an in vivo hollow fiber assay in the dose range 1–6 mg/kg.[13d] The structures of the isolated dichapetalins in the present work are different from those previously reported only in the side chain and the C-6′ substituents. Our results represent the first report on the isolation of dichapetalin triterpenoids with a spiro-lactone side chain from the genus *Phyllanthus*.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on a digital Electrothermal apparatus. Optical rotations were determined on a JASCO DIP 370 digital polarimeter using a 50 mm microcell (1 mL), and CD spectra were recorded in ethanol or methanol on a JASCO J-810 spectropolarimeter. UV spectra were measured in ethanol or methanol on a JASCO 530 spectrometer, and IR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 500 spectrometer in $CDCl_3$, using TMS as internal standard. EIMS were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 or 20 eV (probe). The HRMS were recorded on a Micromass model VQ-TOF-2 spectrometer. Solvents for extraction, chromatography, and recrystallization were distilled prior to use. Silica gel 60 (Merck, 70−230 mesh) and silica gel plates (Merck, Kieselgel $60F_{254}$, 0.5 mm) were used for column chromatography and preparative thin-layer chromatography, respectively.

Plant Material. The aerial parts of *P. acutissima* (Euphorbiaceae) were collected from Ban Fang District, Khon Khean Province of Thailand, in December 2003, and were identified by T. Santisuk. A voucher specimen (BKF no. 129063) of *P. acutissima* has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Extraction and Isolation. The air-dried and finely powdered aerial parts of *P. acutissima* (8.6 kg) were percolated sequentially with hexane (5 × 23 L), EtOAc (7 × 18 L), and MeOH (6 × 17 L) at room temperature. Removal of solvents yielded the hexane, EtOAc, and MeOH extracts in 103, 105, and 443 g quantities, respectively.

The hexane extract (102 g) was subjected to silica gel column chromatography (8.6 kg), eluting with acetone−hexane (0−100%), followed by MeOH−acetone (0−100%) to afford fractions A1−A6 after combination and removal of solvents. Fraction A4 (5.9 g; eluted with 15−17% acetone−hexane) yielded **2** (157.4 mg) after recrystallization from $CHCl_3$−EtOH. Further separation of the residue of fraction A4 (5.8 g) by column chromatography (CH_2Cl_2−hexane and MeOH−CH_2Cl_2 gradients) afforded fractions B1−B4. Fraction B2 (102.6 mg; eluted with 20–40% CH_2Cl_2−hexane) provided **9** (13.7 mg) after preparative TLC (30% EtOAc−hexane; R_f 0.33). Fraction B3 (3.7 g; eluted with 40−100% CH_2Cl_2−hexane and then 2% MeOH−CH_2Cl_2) gave an additional amount of **2** (20.5 mg) after column chromatography (CH_2Cl_2−hexane gradient), followed by preparative TLC (5% MeOH−CH_2Cl_2; R_f 0.71) and recrystallization from $CHCl_3$−EtOH. Fraction A5 (6.8 g; eluted with 20−25% acetone−hexane) gave **1** (393.6 mg) after recrystallization from $CHCl_3$−EtOH. Column chromatography of the residue of fraction A5 (6.6 g) twice (CH_2Cl_2−hexane and acetone−hexane gradients, respectively), followed by recrystallization from $CHCl_3$−EtOH, yielded an additional amount of **1** (169.6 mg). Fraction A6 (7.4 g; eluted with 30−100% acetone−hexane) was purified by column chromatography twice (acetone−hexane gradients), followed by recrystallization from $CHCl_3$−EtOH to afford **5** (32.6 mg).

Separation of the EtOAc extract (104 g) by column chromatography (silica gel, acetone−hexane gradient, followed by MeOH−acetone gradient) afforded fractions A1−A7. Fraction A2 (16.6 g; eluted with 12−20% acetone−hexane) afforded **9** (10.3 mg) after two consecutive column chromatographies (acetone−hexane gradient), followed by preparative TLC (20% acetone−hexane; R_f 0.22) and recrystallization from benzene. Fraction A3 (1.9 g; eluted with 25−30% acetone−hexane) gave **1** (11.0 mg) after addition of $CHCl_3$−EtOH. The residue (1.8 g) was separated by column chromatography (CH_2Cl_2−hexane and MeOH−CH_2Cl_2 gradients) to give fractions B1−B5. Fraction B4 (400 mg; eluted with 0.3% MeOH−CH_2Cl_2) provided **2** (10.1 mg) after column chromatography on Sephadex LH-20 (hexane), followed by recrystallization from $CHCl_3$−EtOH. Fraction B5 (309.4 mg; eluted with 0.5% MeOH−CH_2Cl_2) yielded **4** (8.2 mg) after preparative TLC (MeOH−CH_2Cl_2−hexane, 2:38:60; R_f 0.33). Fraction A4 (9.3 g; eluted with 40−50% acetone−hexane) was further purified by column chromatography (CH_2Cl_2−hexane and MeOH−CH_2Cl_2 gradients) to give fractions C1−C6. Fraction C2 (37.1 mg; eluted with 90% CH_2Cl_2−hexane) yielded **8** (6.1 mg) after recrystallization from $CHCl_3$−EtOH. Fraction C4 (3.5 g; eluted with 1.5% MeOH−CH_2Cl_2) gave **3** (30.2 mg) after column chromatography (MeOH−CH_2Cl_2 gradient) and recrystallization from $CHCl_3$−EtOH. Fraction C5 (2.6 g; eluted with 2% MeOH−CH_2Cl_2) was purified by column chromatography (acetone−hexane gradient) to yield fractions D1−D4. Fraction D2 (979.9 mg; eluted with 15% acetone−hexane) gave an additional amount of **3** (23.0 mg) after addition of $CHCl_3$−EtOH. The residue of fraction D2 (890.2 mg) provided an additional amount of **2** (38.6 mg) after column chromatography (acetone−hexane gradient) and recrystallization from $CHCl_3$−EtOH. Fraction A5 (7.4 g; eluted with 60−80% acetone−hexane) was further purified by column chromatography (MeOH−CH_2Cl_2 gradient) to afford fractions E1−E5. Fraction E3 (2.5 g; eluted with 2–5% MeOH−CH_2Cl_2) afforded **5** (201.5 mg) after recrystallization from $CHCl_3$−EtOH. The residue of fraction E3 (2.1 g) provided additional quantities of **5** (96.6 mg) and **10** (3.2 mg) after column chromatography (MeOH−CH_2Cl_2−hexane gradient) and recrystallization ($CHCl_3$−EtOH for **5** and CH_2Cl_2−EtOH for **10**). Fraction A6 (14.7 g; eluted with 0−10% MeOH−acetone) was purified by column chromatography (MeOH−CH_2Cl_2−hexane gradient) to yield F1−F7. Fraction F5 (6.9 g; eluted with 2:60:38 MeOH−CH_2Cl_2−hexane) gave **7** (12.6 mg) after two consecutive column chromatographic separations (MeOH−CH_2Cl_2 and acetone−hexane gradients), followed by preparative TLC (40% EtOAc−hexane; R_f 0.16) and recrystallization from EtOAc−hexane.

The MeOH extract (442 g) was subjected to column chromatography (CH_2Cl_2−hexane gradient, followed by MeOH−CH_2Cl_2 gradient) to give fractions A1−A6. Fraction A1 (5.9 g; eluted with 0−3% CH_2Cl_2−hexane) yielded **9** (7.8 mg) after two consecutive column chromatographic steps (CH_2Cl_2−hexane and acetone−hexane gradients), followed by preparative TLC (40% EtOAc−hexane; R_f 0.51) and recrystallization from benzene. Fraction A2 (11.7 g; eluted with 3.5–5% MeOH−CH_2Cl_2) was purified by column chromatography (CH_2Cl_2−hexane gradient, followed by MeOH−CH_2Cl_2 gradient) to provide fractions B1−B6. Fraction B3 (3.7 g; eluted with 90−100% CH_2Cl_2−hexane and then 3% MeOH−CH_2Cl_2) yielded **7** (17.5 mg) after two consecutive column chromatographic stages (CH_2Cl_2−hexane and EtOAc−hexane gradients), followed by preparative TLC (30% EtOAc−hexane; R_f 0.10) and recrystallization from CH_2Cl_2−MeOH. Fraction A3 (14.0 g; eluted with 6−15% MeOH−CH_2Cl_2) was further separated by column chromatography (acetone−hexane gradient, followed by MeOH−acetone gradient) to give fractions C1−C10. Fraction C4 (62.0 mg; eluted with 30−35% acetone−hexane) afforded an additional amount of **9** (7.2 mg) after preparative TLC (CH_2Cl_2; R_f 0.56) and recrystallization from EtOAc−hexane. Fraction C10 (6.9 g; eluted with 50−100% MeOH−acetone) yielded **11** (28.9 mg) after column chromatography (acetone−hexane gradient) and recrystallization from CH_2Cl_2−MeOH−H_2O. Fraction A4 (17.0 g; eluted with 17−25% MeOH−CH_2Cl_2) afforded an additional amount of **11** (92.2 mg) after recrystallization from CH_2Cl_2−MeOH−H_2O. The residue of fraction A4 (16.6 g) was further purified by column chromatography (CH_2Cl_2−hexane gradient, followed by MeOH−CH_2Cl_2 gradient) to yield fractions D1−D8. Fraction D4 (591.4 mg; eluted with 90% CH_2Cl_2−hexane) gave **6** (10.7 mg) after preparative TLC (5% MeOH−CH_2Cl_2; R_f 0.36). Fraction D5 (2.8 g; eluted with 7% MeOH−CH_2Cl_2) provided **12** (6.2 mg) after two consecutive column chromatographic steps (CH_2Cl_2−hexane and acetone−hexane gradients), followed by recrystallization from CH_2Cl_2−MeOH−H_2O. Fraction D7 (5.4 g; eluted with 40−60% MeOH−CH_2Cl_2) afforded an additional amount of **11** (18.5 mg) after column chromatography (MeOH−CH_2Cl_2 gradient) and recrystallization from CH_2Cl_2−MeOH−H_2O.

Acutissimatriterpene A (1): colorless needles, mp 232–234 °C; $[\alpha]^{25}_D$ +38.6 (*c* 0.90, $CHCl_3$); CD $\Delta\epsilon_{225}$ +7.46, $\Delta\epsilon_{239}$ +12.12, $\Delta\epsilon_{250}$ +8.61, $\Delta\epsilon_{260}$ +8.73 (3.04×10^{-4} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 231 sh (3.9), 282 (3.6) nm; IR ($CHCl_3$) ν_{max} 3583, 1765, 1657, 1610, 1505, 1491, 1443, 1389, 1329, 1251, 1241 1179, 1056, 1012, 936 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HMBC correlations, Table S1 (Supporting Information); EIMS *m/z* 658 $[M]^+$ (9), 640 $[M - H_2O]^+$ (3), 628 (100), 629 (45), 626 (15), 203 (14); HRTOFMS (ESI positive) *m/z* 681.3447 (calcd for $C_{40}H_{50}O_8Na$ 681.3398).

The *p*-bromobenzoate **1a** was prepared by esterification of **1** with 3 equiv of *p*-ClCOPhBr/DMAP (cat.) in dry CH_2Cl_2 at room temperature for 24 h to afford **1a** in 34% yield. **1a**: colorless needles ($CHCl_3$−EtOH), mp 214–216 °C; $[\alpha]^{27}_D$ +26.4 (*c* 1.18, $CHCl_3$); CD $\Delta\epsilon_{222}$ +3.19, $\Delta\epsilon_{243}$ +15.67, $\Delta\epsilon_{263}$ +2.57, $\Delta\epsilon_{281}$+2.27 (1.16×10^{-4} M, EtOH); HRTOFMS (ESI positive) *m/z* 863.2698 (calcd for $C_{47}H_{53}O_9{}^{79}BrNa$: 863.2765).

Acutissimatriterpene B (2): colorless needles, mp 236–238 °C; $[\alpha]^{25}_D$ +32.5 ($CHCl_3$, *c* 0.98); CD $\Delta\epsilon_{233}$ −1.62, $\Delta\epsilon_{258}$ +6.59 (1.62×10^{-4} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 250 (2.87) nm; IR ($CHCl_3$) ν_{max} 3586, 1765, 1657, 1604, 1495, 1455, 1363, 1329, 1261, 1166, 1074, 1056, 1013, 912 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HMBC correlations, Table S1 (Supporting Information); EIMS *m/z* 614 $[M]^+$ (4), 596 $[M - H_2O]^+$ 584 (100), 585 (42), 470 (19), 198 (14), 168 (14), 129 (19). HRTOFMS (ESI positive) *m/z* 637.3530 (calcd for $C_{39}H_{50}O_6Na$, 637.3500).

Acutissimatriterpene C (3): colorless needles, mp 224–226 °C; $[\alpha]^{26}_D$ −14.5 (*c* 0.98, $CHCl_3$); CD $\Delta\epsilon_{229}$ +6.42, $\Delta\epsilon_{253}$ − 5.02 (9×10^{-5} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 228 sh (4.02), 281 (3.68) nm; IR ($CHCl_3$) ν_{max} 3586, 1767, 1660, 1612, 1505, 1491, 1443, 1389, 1331, 1251, 1161, 1057, 1014, 936 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HMBC correlations, Table S1 (Supporting Information); EIMS *m/z* 658 $[M]^+$ (9), 640 $[M - H_2O]^+$ (11), 628 (100), 514 (33), 443 (25), 242 (54), 135 (93); HRTOFMS (ESI positive) *m/z* 681.3448 (calcd for $C_{40}H_{50}O_8Na$, 681.3398).

By using the procedure described for **1a**, *p*-bromobenzoate **3a** was prepared from **3** in 51% yield. **3a**: colorless needles from $CHCl_3$−EtOH,

mp 204−205 °C; CD $\Delta\epsilon_{223}$ +1.34, $\Delta\epsilon_{242}$ +2.35, $\Delta\epsilon_{260}$ −0.93 (1.16 × 10^{-4} M, EtOH); HRTOFMS (ESI positive) *m/z* 863.2791 (calcd for $C_{47}H_{53}O_9{}^{79}BrNa$, 863.2765).

Acutissimatriterpene D (4): colorless needles, mp 214–215 °C; $[\alpha]^{25}_D$ −31.4 (*c* 0.41, $CHCl_3$); CD $\Delta\epsilon_{220}$ +5.51, $\Delta\epsilon_{245}$ −5.72 (2.1 × 10^{-4} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 253 (3.09) nm; IR ($CHCl_3$) ν_{max} 3586, 1767, 1661, 1605, 1496, 1455, 1389, 1331, 1256, 1162, 1071, 1014, 909 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HMBC correlations, Table S1 (Supporting Information); HRTOFMS (ESI positive) *m/z* 615.3669 $[M + H]^+$ (calcd for $C_{39}H_{51}O_6$, 615.3680).

Acutisssimatriterpene E (5): colorless needles, mp 234–235 °C; $[\alpha]^{27}_D$ +39.3 (*c* 1.04, $CHCl_3$); CD $\Delta\epsilon_{225}$ +4.69, $\Delta\epsilon_{235}$ +9.06, $\Delta\epsilon_{256}$ −0.02, $\Delta\epsilon_{284}$ +1.82 (1.47 × 10^{-4} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 232 (3.82), 282 (3.71) nm; IR ($CHCl_3$) ν_{max} 3571, 1785, 1610, 1505, 1491, 1443, 1390, 1319, 1240, 1182, 1078, 1061, 1042, 989, 966, 935 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HMBC correlations, Table S1 (Supporting Information); EIMS *m/z* 676 $[M]^+$ (12), 658 $[M - H_2O]^+$ (11), 640 $[M - 2H_2O]^+$ (2), 582 (45), 267 (40), 199 (45), 149 (76), 135 (100); HRTOFMS (ESI positive) *m/z* 699.3529 $[M + Na]^+$ (calcd for $C_{40}H_{53}O_9$, 699.3504).

Compound 5a: colorless needles ($CHCl_3$−EtOH), mp 252–254 °C; $[\alpha]^{27}_D$ +27.13 (*c* 1.12, $CHCl_3$); CD $\Delta\epsilon_{224}$ +3.49, $\Delta\epsilon_{236}$ +3.84, $\Delta\epsilon_{259}$ −0.59, $\Delta\epsilon_{289}$+0.32 (1.47 × 10^{-4} M, EtOH); UV (EtOH) λ_{max} (log ϵ) 232 (3.61), 282 (3.49) nm; IR ($CHCl_3$) ν_{max} 3552, 1788, 1611, 1506, 1491, 1443, 1388, 1308, 1240, 1178, 1097, 1071, 1042, 990, 966, 936 cm^{-1}; ^1H NMR ($CHCl_3$, 500 MHz) δ 6.91 (1H, d, J = 1.5 Hz, H-2″), 6.83 (1H, dd, J = 8.0, 1.5 Hz, H-6″), 6.77 (1H, d, J = 8.0 Hz, H-5″), 5.93 (1H, s, H-7″), 5.52 (1H, br d, J = 2.9 Hz, H-15), 5.38 (1H, br d, J = 6.9 Hz, H-2), 4.18 (1H, dd, J = 11.7, 2.6 Hz, H-6′), 4.13 (1H, dd, J = 9.5, 1.4 Hz, H-26a), 4.06 (1H, d, J = 9.5 Hz, H-22), 3.96 (1H, br m, H-7), 3.87 (1H, d, J = 9.5 Hz, H-26b), 3.76 (1H, d, J = 10.7 Hz, H-2′a), 3.58 (1H, d, J = 10.7 Hz, H-2′b), 3.25 (3H, s, OCH_3-25), 2.83 (1H, t, J = 9.1 Hz, H-17), 2.59 (1H, obsc., H-5′a), 2.52 (1H, obsc., H-16a), 2.49 (1H, dd, J = 14.4, 1.4 Hz, H-24a), 2.34 (1H, obsc., H-12a), 2.31 (1H, d, J = 14.4 Hz, H-24b), 2.26 (1H, obsc., H-16b), 2.14 (1H, dd, J = 13.3, 2.6 Hz, H-5′b), 2.11 (1H, obsc., H-20), 2.03 (1H, obsc., H-9), 1.98 (1H, obsc., H-5), 1.94 (1H, dd, J = 16.3, 6.9 Hz, H-1a), 1.88 (1H, br m, H-6a), 1.72 (1H, obsc., H-1b), 1.70 (1H, obsc., H-11a), 1.67 (1H, dd, J = 13.9, 2.4 Hz, H-6b), 1.58 (1H, br m, H-11b), 1.45 (1H, obsc., H-12), 1.42 (3H, s, H-27), 1.31 (3H, s, H-28), 1.09 (3H, s, H-30), 1.09 (3H, s, H-18), 1.02 (3H, s, H-19); ^{13}C NMR ($CHCl_3$, 125 MHz) δ 174.3 (s, C-21), 161.7 (s, C-14), 147.6 (s, C-3″), 146.8 (s, C-4″), 139.4 (s, C-3), 136.8 (s, C-1″), 119.3 (d, C-15), 119.2 (d, C-6″), 118.3 (d, C-2), 111.9 (s, C-23), 108.0 (d, C-5″), 106.6 (d, C-2″), 100.9 (t, C-7″), 82.7 (s, C-25), 81.6 (d, C-6′), 76.8 (t, C-26), 75.7 (d, C-22), 72.7 (t, C-2′), 71.7 (d, C-7), 53.5 (d, C-20), 50.6 (q, OCH_3-25), 47.1 (s, C-13), 46.2 (d, C-17), 45.4 (t, C-24), 44.4 (d, C-5), 44.1 (s, C-8), 40.7 (t, C-5′), 40.2 (d, C-9), 39.2 (t, C-1), 38.1 (s, C-4), 36.9 (s, C-10), 33.4 (t, C-16), 32.3 (t, C-12), 27.2 (q, C-18), 23.8 (q, C-28), 23.3 (t, C-6), 19.4 (q, C-30), 18.8 (q, C-27), 16.1 (t, C-11), 16.0 (q, C-19); HMBC correlations, Table S1 (Supporting Information); EIMS *m/z* 676 $[M]^+$ (21), 658 $[M - H_2O]^+$ (4), 640 $[M - 2H_2O]^+$ (0.4), 582 (69), 251 (35), 199 (52), 149 (61), 135 (100); HRTOFMS (ESI positive) *m/z* 699.3506 $[M + Na]^+$ (calcd for $C_{40}H_{53}O_9Na$, 699.3504).

By using the procedure described for **1a**, the *p*-bromobenzoate derivatives **5b** and **5c** were prepared from **5a** in 54 and 15% yields, respectively. **5b**: colorless needles ($CHCl_3$−EtOH), mp 238–239 °C; $[\alpha]^{25}_D$ +10.8 (*c* 0.5, $CHCl_3$); CD $\Delta\epsilon_{228}$ +2.50, $\Delta\epsilon_{247}$ +1.93, $\Delta\epsilon_{257}$ +2.93 (2.32 × 10^{-4} M, EtOH); HRTOFMS (ESI positive) *m/z* 881.2845 (calcd for $C_{47}H_{55}O_{10}{}^{79}BrNa$, 881.2871). **5c***:* mp 209–210 °C; $[\alpha]^{27}_D$ +11.7 (*c* 0.56, $CHCl_3$); CD $\Delta\epsilon_{224}$ +2.00, $\Delta\epsilon_{242}$ +5.34, $\Delta\epsilon_{255}$ −0.90, $\Delta\epsilon_{283}$ +1.15 (9.59 × 10^{-4} M, EtOH); HRTOFMS (ESI positive) *m/z* 1063.2239 (calcd for $C_{54}H_{58}O_{11}{}^{79}Br_2Na$, 1063.2238).

4-*O*-(2-*O*-Methyl-α-L-arabinopyranosyl)diphyllin (acutissimaligNan A) (6): white powder, mp 177–178 °C; $[\alpha]^{24}_D$ +12.1 (*c* 0.54, $CHCl_3$); CD $\Delta\epsilon_{228}$ −8.39, $\Delta\epsilon_{239}$ +28.02, $\Delta\epsilon_{261}$ −18.92, $\Delta\epsilon_{274}$ +5.25, $\Delta\epsilon_{318}$ −12.78, $\Delta\epsilon_{353}$ +6.19 (5 × 10^{-4} M, MeOH); UV λ_{max} (log ϵ) 220 (3.61), 257 (3.94), 293 (3.26), 314 (3.26), 350 (2.92) nm; IR ($CHCl_3$) ν_{max} 3566, 1758, 1625, 1600, 1507 1481, 1456, 1435, 1390, 1338, 1264, 1168, 1072, 1042, 1014, 937 cm^{-1}; ^1H NMR ($CHCl_3$, 500 MHz) δ 7.80 (1H, s, H-5); 7.10 (1H, s, H-8), 6.96 (1H, d, J = 7.9 Hz, H-5′), 6.79–6.85 (2H, overlapping signals, H-2′ and H-6′), 6.10 (1H, br s, H-7″a), 6.054/6.052* (1H, d each, J = 1.6 Hz, H-7″b), 5.571/5.565* (1H, d each, J = 15.1 Hz, H-3aa), 5.459/5.455* (1H, d each, J = 15.1 Hz, H-3ab), 4.79 (1H, d, J = 7.2 Hz, H-1″), 4.07 (3H, s, OCH_3-6), 4.049/4.045* (1H, dd each, J = 13.0, 1.8 Hz, H-5″a), 3.99 (1H, br m, H-4″), 3.90 (3H, s, OCH_3-2″), 3.82 (3H, s, OCH_3-7), 3.74 (1H, dd, J = 9.1, 3.3 Hz, H-3″), 3.69 (1H, dd, J = 9.1, 7.2 Hz, H-2″), 3.44 (1H, br d, J = 13.0 Hz, H-5″b); ^{13}C NMR ($CHCl_3$, 125 MHz) δ 170.7 (s, C-2a); 152.9 (s, C-6), 151.1 (s, C-7), 148.5 (s, C-3′ and C-4′), 145.1 (s, C-4), 137.4 (s, C-1), 132.09/132.05* (s each, C-3), 131.8 (s, C-8a), 129.2 (s, C-1′), 127.8 (s, C-4a), 124.5/124.4* (d each, C-6′), 120.2 (s, C-2), 111.6/111.5* (d each, C-2′), 109.13/109.10* (d each, C-5′), 107.3 (d, C-8), 106.1 (d, C-1″), 102.1 (t, C-7′), 101.3 (d, C-5), 82.5 (d, C-2″), 73.9 (d, C-3″), 69.1 (d, C-4″), 68.4 (t, C-3a), 67.0 (t, C-5″), 62.6 (q, OCH_3-2″), 57.0 (q, OCH_3-6), 56.8 (q, OCH_3-7); EIMS *m/z* 380 [M − sugar]$^+$ (100), 321 (11), 293 (20); HRTOFMS (ESI positive) *m/z* 527.1542 $[M + H]^+$ (calcd for $C_{27}H_{27}O_{11}$, 527.1548).

(2*E*,3*S*)-2-(4-Hydroxy-3-methoxybenzylidene)-3-(4-hydroxy-3-methoxybenzyl)butyrolactone (acutissimaligNan B) (7): white powder, mp 128–129 °C; $[\alpha]^{25}_D$ +29.8 (*c* 0.63, $CHCl_3$); CD $\Delta\epsilon_{224}$ −1.16, $\Delta\epsilon_{242}$ +1.15, $\Delta\epsilon_{252}$ +1.00, $\Delta\epsilon_{299}$ +2.83, $\Delta\epsilon_{353}$ +0.72 (2.25 × 10^{-4}, EtOH); UV (EtOH) λ_{max} (log ϵ) 229 (3.46), 286 (3.28), 329 (3.51) nm; IR ($CHCl_3$) ν_{max} 3539, 1743, 1646, 1606, 1596, 1516, 1465, 1453, 1432, 1359, 1274, 1252, 1181, 1159, 1124, 1035, 909 cm^{-1}; ^1H NMR ($CHCl_3$, 500 MHz) δ 7.52 (1H, d, J = 1.8 Hz, H-6), 7.21 (1H, dd, J = 8.3, 1.9 Hz, H-6′), 7.03 (1H, d, J = 1.9 Hz, H-2′), 6.99 (1H, d, J = 8.3 Hz, H-5′), 6.86 (1H, d, J = 8.0 Hz, H-5″), 6.71 (1H, dd, J = 8.0, 1.9 Hz, H-6″), 6.64 (1H, d, J = 1.9 Hz, H-2″), 4.27 (2H, app. d, J = 4.2 Hz, H-4a, H-4b), 3.92 (3H, s, OCH_3-3′), 3.86 (3H, s, OCH_3-3″), 3.81 (1H, m, H-3), 3.06 (1H, dd, J = 14.5, 4.3 Hz, H-5a), 2.63 (1H, dd, 14.5, 10.0 Hz, H-5b); ^{13}C NMR ($CHCl_3$, 125 MHz) δ 172.6 (s, C-1), 147.7 (s, C-4′), 146.8 (s, C-3′), 146.7 (s, C-3″), 144.7 (s, C-4″), 137.4 (d, C-6), 129.7 (s, C-1″), 126.6 (s, C-1′), 125.7 (s, C-2), 124.0 (d, C-6′), 121.3 (d, C-6″), 115.0 (d, C-5′), 114.7 (d, C-5″), 112.7 (d, C-2′), 111.6 (d, C-2″), 69.7 (t, C-4), 56.0 (q, OCH_3-3′), 55.9 (q, OCH_3-3″), 39.7 (d, C-3), 37.5 (t, C-5); EIMS *m/z* 356 $[M]^+$ (6), 219 (26), 159 (16), 138 (11), 137 (100), 131 (15), 103 (10); HRTOFMS (ESI positive) *m/z* 379.1161 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_6Na$, 379.1152).

X-ray Crystal Data of 1a and 5b. X-ray crystallographic data of compounds **1a** and **5b** were collected at room temperature on a Bruker-Nonius kappaCCD diffractometer with Mo Kα radiation (λ = 0.71073 Å). The crystal structures were solved by direct methods using SIR97, and then all atoms except hydrogen atoms were refined anisotropically on F^2 using SHELXL-97. Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited with the Cambridge Crystallographic Data Center (CCDC 622798 and 622799). These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Compound 1a: $C_{47}H_{53}BrO_9$, MW 841.84, orthorhombic, $P2_12_12_1$, a = 7.8919(4) Å, b = 12.0853(9) Å, c = 43.5920(30) Å, V = 4157.6(5) Å^3, Z = 4, μ = 1.045 mm^{-1}, D_x = 1.345 g/cm^{-3}, $F(000)$ = 1768, ω–2θ scans, θ_{max} = 21.99°, reflections collected = 8557, unique reflections = 3873 (R_{int} = 0.062). For the final refinement [$I > 2\sigma(I)$], R_1 = 0.0476, wR_2 = 0.1106, GOF = 1.050, and Flack parameter = 0.006(15), 0.94(2) for the opposite enantiomer.

Compound 5b: $C_{47}H_{55}BrO_{10}$, MW 859.85, monoclinic, $P2_1$, a = 10.1863(5) Å, b = 14.2352(10) Å, c = 14.5801(8) Å, β = 97.652(4)°, V = 2095.3(2) Å^3, Z = 2, μ = 1.040 mm^{-1}, D_x = 1.363 g/cm^{-3}, $F(000)$ = 904, ω–2θ scans, θ_{max} = 24.98°, reflections collected = 9685, unique reflections = 3598 (R_{int} = 0.052). For the final refinement [$I > 2\sigma(I)$], R_1 = 0.0459, wR_2 = 0.1136, GOF = 1.029, and Flack parameter = −0.01 (1), 0.86(1) for the opposite enantiomer.

Cytotoxicity Assay. The cytotoxic activities of all tested compounds were carried out using the in vitro sulforhodamine B (SRB) method.[19] Test samples were dissolved in DMSO as a stock concentration at 20 μg/mL and were tested in triplicate with a final concentration of DMSO at 0.5%. The cancer cell lines were grown in a 96-well tissue culture plate in the following media: P-388, in Fisher's medium with 5% bovine calf serum (BCS); KB, in DMEM (Dulbecco's modified Eagle medium) with 10% BCS; Col-2 and ASK, in MEM with 10% fetal bovine serum (FBS); MCF-7 and Lu-1, in MEM (minimum essential medium with Earle's salt with L-glutamine) with 5% BCS. After 72 h of drug exposure at 37 °C, 5% CO_2 in air, and 100% relative humidity, cells were fixed with a final concentration of 10% trichloroacetic acid and stained with 0.4% sulforhodamine B in 1% acetic acid. After removal of the unbound dye by washing, the bound and dried stain was

solubilized with 10 mM trizma base. The absorbance at wavelength 515 nm was read on a Fluostar optima BMG plate reader. The cytotoxic activity is expressed as 50% effective dose (ED_{50}).

Cell-Based Assay for Anti-HIV-1 Activity. The combination of syncytium and colorimetric cytotoxicity assays employing ΔTat/Rev defective HIV-1 ($^{\Delta Tat/Rev}$MC99) and 1A2 cells system were used.[20] Cells were seeded into a 96-well tissue culture plate, followed by serial 2-fold dilutions of the compound, in triplicate, and incubated for an hour before the addition of virus. 3′-Azido-5′-thymidine (AZT) was used as a positive control of HIV-1 inhibitor. Virus control wells contained cells and the virus only and cell control wells contained neither extract nor virus; cytotoxicity control wells contained cells with the extract or compound. The result was expressed as the effective concentration of the drug that reduced syncytium formation by 50% (EC_{50}). The same batch of 1A2 cells was used for the colorimetric cytotoxicity assay that was carried out in parallel. The procedure was similar to the syncytium assay, but the virus was replaced by medium and tested in duplicate wells. Control wells included medium, drug, and cell control. After incubation for three days, XTT tetrazolium-phenazine methosulfate solution was added to each well. After the soluble formazan developed, the optical density at A_{450} was measured with a reference at A_{650}. The results are expressed as 50% inhibitory concentrations (IC_{50}), i.e., the doses that inhibit 50% metabolic activity of uninfected cells.

Anti-HIV-1 RT Assay. The method previously described[21] was used for testing RT inhibition. Tannin-free compounds, final concentration 200 μg/mL in 10% DMSO, was added to the reaction mixture prior to the addition of HIV-1 RT (Amersham). Control assay was performed without the compounds or extracts, but with an equivalent volume of 10% DMSO. The non-nucleoside reverse transcriptase inhibitor nevirapine (2 μg/mL) was used as a positive control. The results from duplicate wells were averaged and the percent RT inhibition was calculated.

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Supporting Information Available: HMBC correlations observed for triterpenes **1**–**5** and **5a** and for lignans **6** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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