Synthesis and Cytotoxicity of Bidesmosidic Betulin and Betulinic Acid Saponins

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The naturally occurring cytotoxic saponin 28-*O*-β-D-glucopyranosylbetulinic acid 3β-*O*-α-L-arabinopyranoside (**3**) was easily synthesized along with seven bidesmosidic saponins starting from the lupane-type triterpenoids betulin (**1**) and betulinic acid (**2**). As highlighted by the preliminary cytotoxicity evaluation against A549, DLD-1, MCF7, and PC-3 human cancer cell lines, the bidesmosidic betulin saponin **22a**, bearing α-L-rhamnopyranoside moieties at both C-3 and C-28 positions, was determined to be a potent cytotoxic agent (IC_{50} 1.8−1.9 μM).

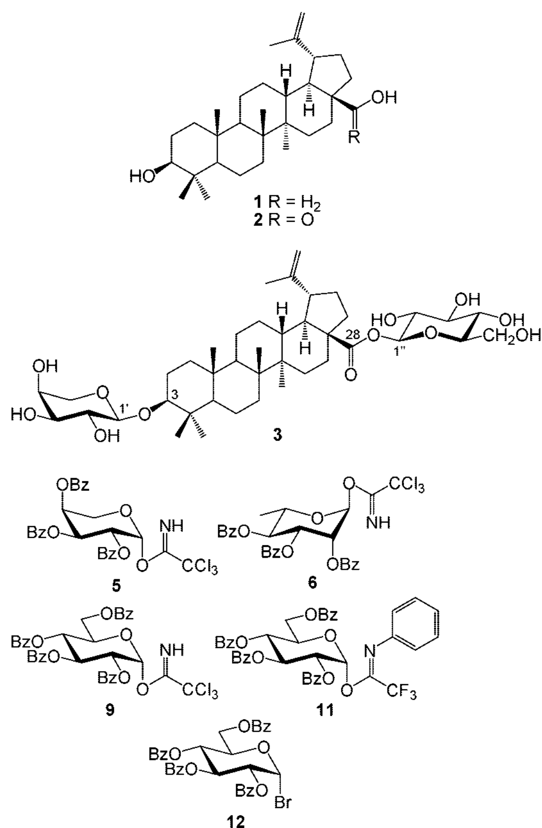
Bidesmosidic saponins are naturally occurring compounds that consist of a triterpenoid or steroid aglycone bearing two sugar moieties usually at the C-3 and C-28 positions.[1] Biological activities exhibited by saponins are quite diversified (cytotoxic, antitumor, anti-inflammatory, molluscicidal) and have been reviewed extensively.[2] However, clinical development of saponins as pharmacological agents is strongly hampered because of their hemolytic activity, inducing toxicity in most animals when delivered intravenously.[3] Interestingly, it has been reported that bidesmosidic saponins are considerably less hemolytic compared to monodesmosides[4] and thus represent attractive chemical targets for structure−activity relationship (SAR) studies.

The first synthesis of a bidesmosidic saponin was achieved by the group of Biao Yu[5] in 1999. Since this accomplishment, several syntheses of bidesmosides have been published, although most of them are solely related to diosgenin[6−8] or oleanolic acid[5,9−12] as aglycones. Betulin (**1**) and betulinic acid (**2**) are cytotoxic lupane-type triterpenoids widely distributed in nature.[13,14] Synthesis of monodesmosidic lupane-type saponins has been reported by us[15,16] and by other groups.[17−22] However, to our knowledge, the only example of the synthesis of betulinic acid bidesmosides is the preparation of the 3,28-bis-β-D-glucopyranoside derivative.[20] Natural bidesmosidic saponins of the lupane-type are scarce and have been isolated principally from plant species of the *Schefflera*[23−25] and *Pulsatilla*[26−28] genera. Braca and co-workers[25] isolated the 3β-*O*-(α-L-arabinopyranosyl)lup-20(29)-ene-28-*O*-β-D-glucopyranosyl ester (**3**) from the aerial parts of *S. rotundifolia*, a plant used as a folk remedy in Asian countries. Bidesmosidic saponin **3** exhibited noticeable cytotoxic activity against J774.A1, HEK-293, and WEHI-164 cell lines and was found, in this study, to be more active than glycosides having oleanolic acid or hederagenin as aglycones.

We now report the synthesis of the natural bidesmosidic betulinic acid saponin **3** along with seven other bidesmosides (**16a**, **16b**, **19**, **21a**, **21b**, **22a**, and **22b**) containing D-glucose, L-rhamnose, and L-arabinose moieties starting from the parent triterpenoids betulin (**1**) and betulinic acid (**2**). The *in vitro* cytotoxic activity of the synthesized saponins was evaluated against human cancer cell lines (A549, DLD-1, MCF7, and PC-3).

Results and Discussion

In order to synthesize bidesmosidic betulin saponins, we first planned to introduce arabinopyranosyl or rhamnopyranosyl moieties at the C-3 position of **1** prior to glucosylating the C-28 position. As depicted in Scheme 1, betulin (**1**)[15] was treated with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in conjunction with imidazole and 4-dimethylaminopyridine (DMAP) in refluxing tetrahydrofuran (THF) to give **4** (90%) protected at the C-28 primary hydroxyl position.[7] The latter was glycosylated with the known 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl trichloroacetimidate (**5**)[5] or 2,3,4-tri-*O*-α-L-rhamnopyranosyl trichloroacetimidate (**6**)[29] under the promotion of the Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dry CH_2Cl_2 at room temperature to afford protected monodesmosides **7a** and **7b** in yields of 71% and 76%, respectively. Desilylation of **7a** and **7b** under standard conditions,[7] i.e., tetrabutylammonium bromide (TBAF) and acetic acid (HOAc) in refluxing THF, readily furnished benzoylated betulin saponins **8a** (75%) and **8b** (87%). Since the next step consisted in the glucosylation at the C-28 position, we tried to couple 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**9**)[30] with **8a** using the above-mentioned glycosylation conditions. However, the reaction afforded the rearrangement product allobetulin 3β-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside in 42% yield with no trace of the desired bidesmosidic glycoside **10a**. Similar treatment of the acceptor **8b** with the donor **9** led to the exclusive formation of



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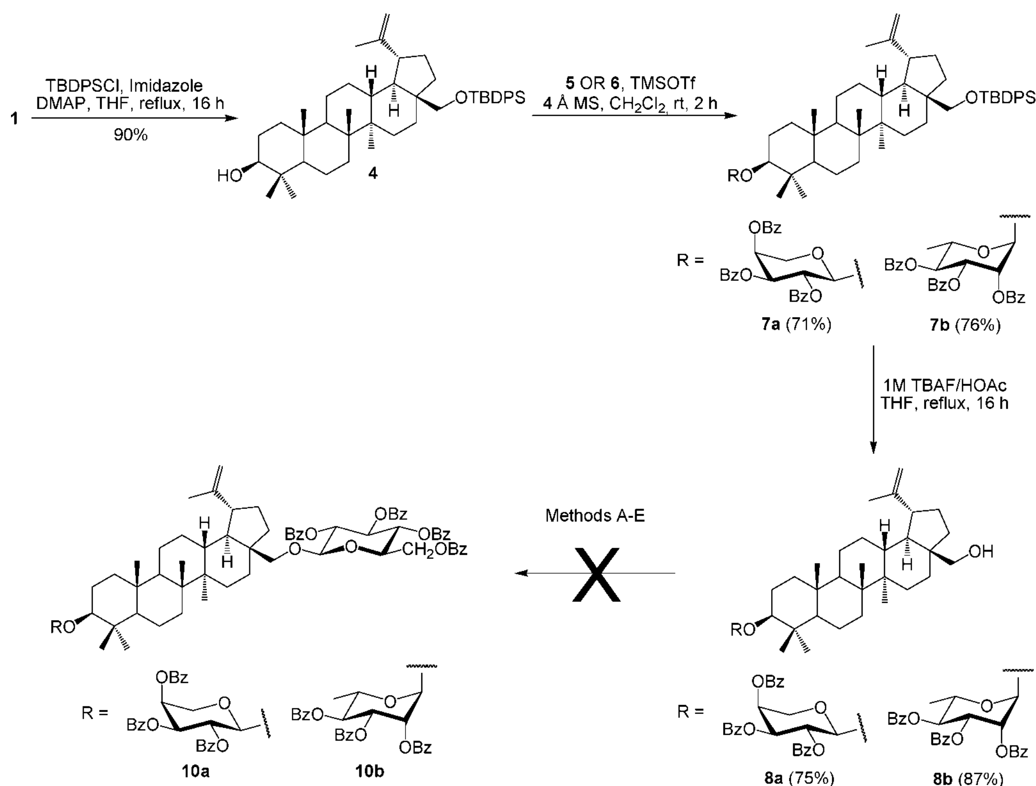
Scheme 1. Attempts to Synthesize Bidesmosidic Betulin Saponins (**10a** and **10b**)[a]



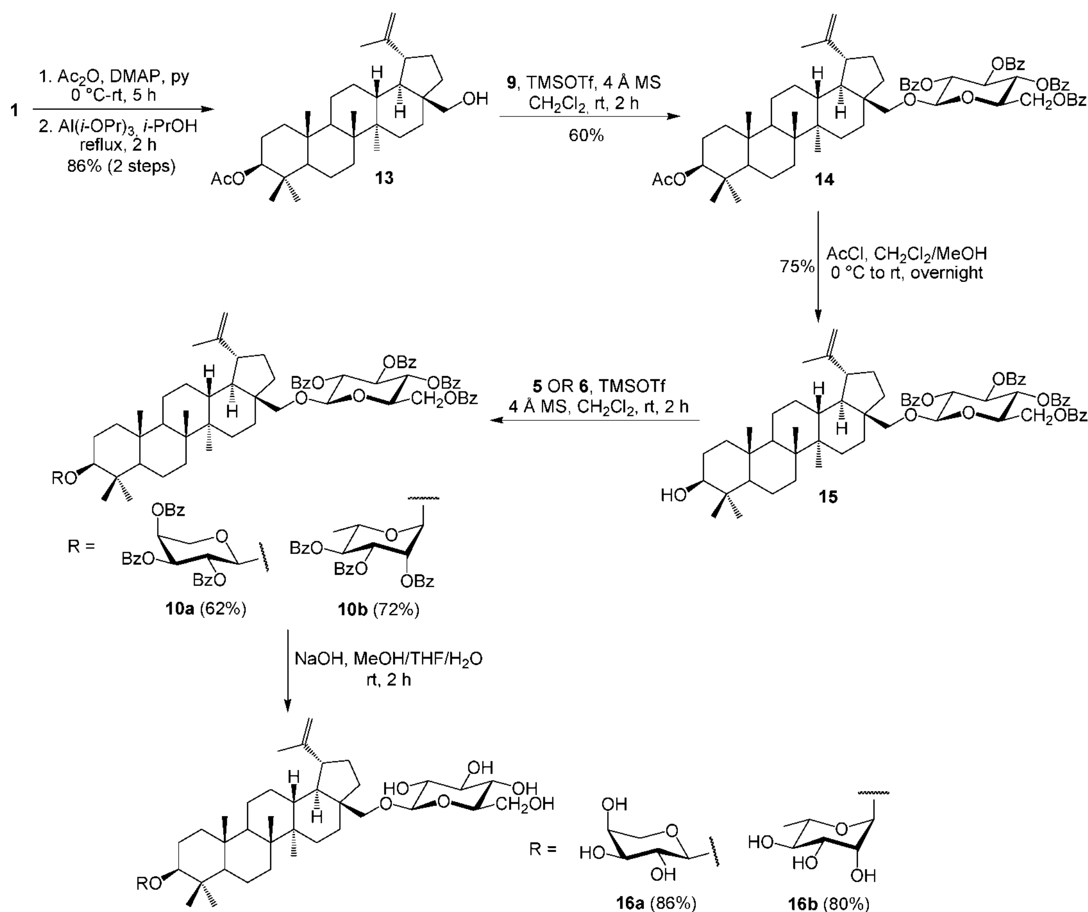
[a] A: donor **9** (1.5 equiv), TMSOTf, CH_2Cl_2, 4 Å molecular sieves, rt, 16 h; B: inverse procedure, donor **9** (1.5 equiv), TMSOTf, CH_2Cl_2, 4 Å molecular sieves, −10 °C to rt, 2.5 h; C: donor **12** (1.5 equiv), AgOTf, CH_2Cl_2, 4 Å molecular sieves, −78 to 0 °C, 2 h; D: donor **11** (1.5 equiv), $BF_3 \cdot OEt_2$, CH_2Cl_2, 4 Å molecular sieves, −78 to 0 °C, 24 h; E: donor **12** (1.3 equiv), K_2CO_3, Bu_4NBr, CH_2Cl_2/H_2O 1:1, reflux, 5 h.

the *trans*-esterification product 28-*O*-benzoylbetulin 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside in 42% yield. As shown in Scheme 1, further modifications of the glycosylation conditions were considered using the acceptor **8b** in conjunction with various glucosyl donors (**9**, **11**, and **12**) and promoters such as boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) and silver trifluoromethanesulfonate (AgOTf). Both Schmidt's inverse procedure[31] and phase-transfer conditions[32]were also tried in order to glucosylate the C-28 position of **8b**. Unfortunately, all these attempts failed to yield the target bidesmoside **10b**. Instead, rapid decomposition of sugar donor (**9**, **11**, and **12**) was generally observed on the basis of TLC analysis. It is worth noting that **8b** was nearly quantitatively transformed into allobetulin 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside when the Lewis acid AgOTf was used as promoter of the glycosylation reaction. The yields of the rearrangement were comparable to those reported by Li and co-workers for the preparation of allobetulin from betulin (**1**) catalyzed by solid acids.[33]

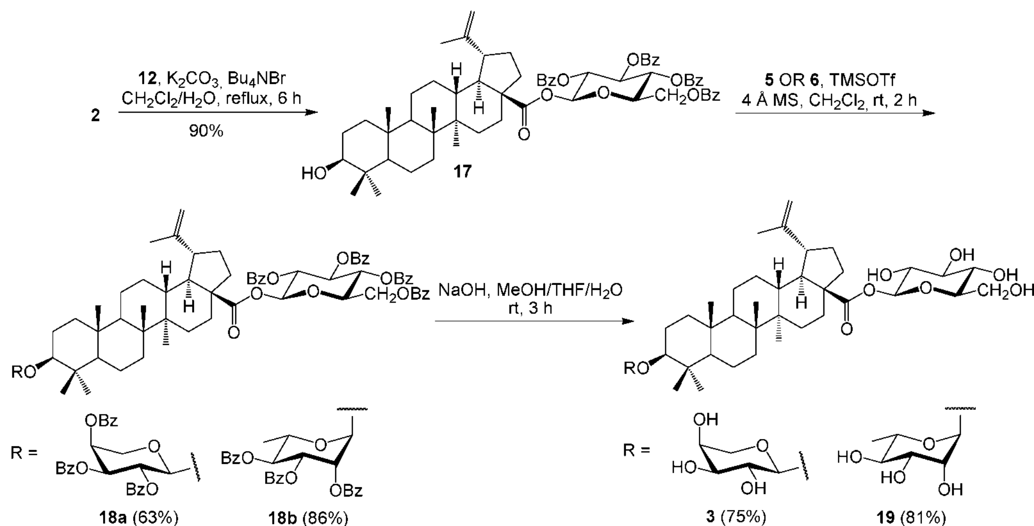
Therefore, we turned to another approach for the synthesis of bidesmosidic betulin saponins. According to Scheme 2, the known betulin 3-acetate (**13**)[16] was prepared in good yield (86%, two steps) from **1** following a reported procedure. Once again, attempts to glucosylate the acceptor **13** with **9** under the catalytic action of TMSOTf (0.1 equiv) in dry CH_2Cl_2 20 mL $mmol^{-1}$ afforded rearrangement products (allobetulin 3-acetate, 30% yield) and *trans*-esterification (28-*O*-benzoylbetulin 3-acetate, 17% yield) instead of the desired glycoside **14**. However, condensation of **13** and **9** proceeded smoothly to furnish **14** (60% yield) when only 0.05 equiv of TMSOTf was used in 40 mL $mmol^{-1}$ of dry CH_2Cl_2. Thereafter, deacetylation of the C-3 position was achieved by treatment of **14** with acetyl chloride (AcCl)[34] in dry CH_2Cl_2/MeOH (1:2) to afford **15** in good yield (75%). The latter acceptor was coupled with the donor **5** or **6** using TMSOTf as the promoter to give the fully benzoylated bidesmosides **10a** (62%) and **10b** (72%), which were deprotected using standard conditions (NaOH, MeOH/THF/H_2O, 1:2:1) to provide the target bidesmosidic betulin saponins **16a** and **16b** in excellent yields (86% and 80%, respectively). The overall yields for the syntheses were 24% for **16a** and 26% for **16b** over four linear steps starting from betulin 3-acetate (**13**).

Synthesis of the natural bidesmosidic betulinic acid saponin **3** along with the non-natural saponin **19** was achieved in a straightforward manner. As depicted in Scheme 3, the lupane-type triterpenoid betulinic acid (**2**) was condensed with the known donor 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**12**)[35] under phase-transfer conditions[32] using K_2CO_3 and TBAF in a refluxing solution of CH_2Cl_2/H_2O (1:1) to furnish **17** in excellent yield (90%). The latter was coupled with the donor **5** or **6** under the promotion of TMSOTf to afford **18a** (63%) and **18b** (86%). Subsequent removal of the benzoyl groups by treatment with NaOH in MeOH/THF/H_2O provided the target bidesmosidic saponins **3** (75%) and **19** (81%). The overall yields for the syntheses were 43% for **3** and 63% for **19** over three linear steps starting from **1**. Unexpectedly, it was found that the physical and analytical data (^1H NMR, ^{13}C NMR, and $[\alpha]_D$) of saponin **3** were not in agreement with those reported for the natural product isolated from *S. rotundifolia*.[25] Indeed, under the same NMR experimental conditions (300 K, MeOD), the chemical shifts and coupling constants of the sugar moieties of saponin **3** were different from those of the isolated compound (Table 1). Recently, we also found such differences in NMR spectral data during the synthesis of a structurally similar betulinic acid saponin isolated by Braca and co-workers.[36] High-resolution electrospray ionization mass spectra (HRESIMS) and extensive 1D and 2D NMR analyses (^1H, ^{13}C, DEPT-135, COSY, TOCSY, HSQC, and HMBC) further proved that the structure of the synthetic saponin **3** was correct.

Scheme 2. Completion of the Synthesis of Bidesmosidic Betulin Saponins (**16a** and **16b**)



Scheme 3. Synthesis of Bidesmosidic Betulinic Acid Saponins (**3** and **19**)



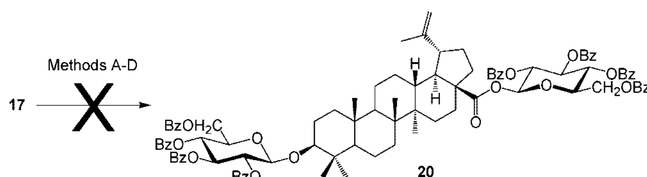
Surprisingly, glucosylation at the C-3 position of 28-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosylbetulinic acid (**17**) proved to be very difficult. In fact, as shown in Scheme 4, all attempts to condense the acceptor **17** with either the trichloroacetimidate sugar donor **9** under Schmidt's normal[37] and inverse procedure[31] or the bromide sugar donor **12** in conjunction with silver oxide (Ag_2O)[38] and AgOTf[39] (modified Koenigs−Knorr methods) failed to yield the fully protected bidesmosidic betulinic acid saponin **20**. According to TLC and NMR analysis, no coupling product was observed in any assays and the acceptor **17** was nearly fully recovered. Thus, we chose to adopt another strategy in which the unprotected betulin (**1**) and betulinic acid (**2**) are glycosylated at both C-3 and C-28 positions via Schmidt's inverse procedure[31] (Scheme 5). Using this methodology, the acceptors (**1** or **2**) and the promoter (TMSOTf) were premixed before the dropwise addition of the sugar donors (**6** or **9**, 3 equiv) at low temperature (−10 °C). Deprotection of the crude product (NaOH, MeOH/THF/H_2O) and purification by C-18 inversed phase flash chromatography afforded the target saponins (**21a**, **21b**, **22a**, and **22b**) in yields ranging from 37% to 84% over two steps. As expected, the 1,2-*trans*-glycosidic linkage (α-L-rhamnoside and β-D-glucoside) of saponins was clearly proved by ^{1}H NMR analysis (δ 4.98, d, $J_{1,2}$ 7.8 Hz and δ 4.30, d, $J_{1,2}$ 7.6 Hz, H-1′ for **21a** and **21b**; δ 4.76, br s and δ 4.72, d, $J_{1,2}$ 1.3 Hz, H-1′ for **22a** and **22b**).[40]

Table 1. Comparison of ^{1}H and ^{13}C NMR Spectral Data (300 K, MeOD, sugar moieties) and $[\alpha]^{25}_{D}$ between Synthetic and Isolated Saponin **3**

sugar (position)	synthetic saponin[a] δ_H (ppm)	J (Hz)	δ_C (ppm)	isolated saponin[b] δ_H (ppm)	J (Hz)	δ_C (ppm)
Ara (1)	4.26	d (6.6)	107.1	4.50	d (6.8)	105.2
Ara (2)	3.54	m	72.8	3.50	dd (8.5,6.8)	72.4
Ara (3)	3.50	m	74.3	3.66	dd (8.5, 3.0)	75.2
Ara (4)	3.79	m	69.5	4.02	m	70.5
Ara (5a)	3.81	m	66.3	3.90	dd (12.0, 2.0)	66.0
Ara (5b)	3.51	m		3.60	dd (12.0, 3.0)	
Glc (1)	5.49	d (8.1)	95.2	5.40	d (7.5)	95.6
Glc (2)	3.32	m	74.1	3.42	dd (9.0, 7.5)	74.2
Glc (3)	3.37	m	78.4	3.49	t (9.0)	77.9
Glc (4)	3.37	m	71.1	3.39	t (9.0)	71.0
Glc (5)	3.37	m	78.8	3.41	m	78.1
Glc (6a)	3.84	m	62.4	3.87	dd (12.0, 3.0)	62.2
Glc (6b)	3.70	m		3.61	dd (12.0, 5.0)	
$[\alpha]^{25}_{D}$	+12.2 (*c* 0.1, MeOH)			+93 (*c* 0.1, MeOH)		

[a] Synthetic product of the present work (NMR 400 MHz). [b] Spectral data of ref 25 (NMR 600 MHz).

Scheme 4. Attempts to Synthesize Benzoylated Bidesmosidic Betulinic Saponins (**20**)[a]



[a] A: donor **9** (1.5 equiv), TMSOTf, CH_2Cl_2, 4 Å molecular sieves, rt, 16 h; B: inverse procedure, donor **9** (3 equiv), TMSOTf, CH_2Cl_2, 4 Å molecular sieves, −10 °C to rt, 3.5 h; C: donor **12** (1.5 equiv), Ag_2O, CH_3CN/CH_2Cl_2, 4 Å molecular sieves, rt, 4 days; D: donor **12** (1.5 equiv), AgOTf, CH_2Cl_2, 4 Å molecular sieves, 0 to 16 °C, 2 h.

In vitro cytotoxic activity of bidesmosidic saponins was evaluated against four human cancer cell lines including lung carcinoma (A549) and colorectal (DLD-1), breast (MCF7), and prostate (PC-3) adenocarcinomas.[41] The parent triterpenoids betulin (**1**)[15] and betulinic acid (**2**)[42] and the clinically used etoposide were used as positive controls. The cytotoxicity of 28-*O*-*β*-D-glucopyranosides of betulin[15] and betulinic acid[18] was also investigated. Moreover, cytotoxic activity was assessed against human normal skin fibroblasts (WS1), but no selectivity was observed for the new series of bidesmosidic saponins.

It had been shown in previous structure−activity relationship (SAR) studies that the free C-28 carboxylic acid function is important to preserve the cytotoxicity of betulinic acid (**2**).[18,43−45] As revealed in Table 2, in our SAR study, this assertion was verified for the two monodesmosidic betulin and betulinic acid saponins bearing a single glucopyranoside moiety at C-28 (IC_{50} >100 μM). On the other hand, the cytotoxicity profile of most of the synthesized bidesmosidic saponins bearing an additional sugar moiety at C-3 was generally similar or higher than betulinic acid (**2**) against tested cancer cell lines. Bidesmosidic saponins **21a** and **21b** were the sole exceptions to this general tendency since the presence of *β*-D-glucopyranoside moieties at both C-3 and C-28 positions seems to have a detrimental effect on cytotoxicity. Nevertheless, saponins **21a** and **21b** were preferentially cytotoxic and significantly ($P < 0.05$) more active than betulinic acid (**2**) against breast adenocarcinoma (MCF7) cells (IC_{50} 14.5 and 20 μM, respectively).

It is noteworthy that the natural bidesmosidic betulinic acid saponin **3**, which features an α-L-arabinopyranoside moiety at C-3, was only moderately cytotoxic against the cancer lines (IC_{50} 23−76 μM), whereas the betulin analogue **16a**, bearing the same sugar residues, was more cytotoxic than betulinic acid (**2**) against MCF7 and PC-3 cell lines (IC_{50} 9.5 and 5.3 μM, respectively).

In this SAR study, the most active saponins were generally those bearing α-L-rhamnopyranoside moieties. Indeed, bidesmosides **16b**, **19**, **22a**, and **22b** inhibited the growth of human cancer cell lines with IC_{50} values ranging from 1.7 to 23 μM. Saponins **22a** and **22b**, containing an α-L-rhamnopyranoside moiety at both C-3 and C-28 positions, were highly cytotoxic against all tested cancer cell lines (IC_{50} 1.7−1.9 and 6.0−7.2 μM, respectively) and significantly more active than their parent triterpenes ($P < 0.05$). Notably, bidesmosidic betulin saponin **22a** was the most potent of all tested compounds to inhibit the growth of human cancer cell lines. The increase in cytoxicity correlated with the presence of rhamnose moieties was also reported in the literature for solasodine steroidal glycosides.[46,47] It was suggested that certain types of cancer cells may have protein receptors, such as lectins,[48−50] that recognize rhamnose moieties and facilitate movement of the drug into the cellular cytoplasm.[46] Thus, these rhamnose receptors could serve to deliver the anticancer agent directly to the tumor.[51−53]

In summary, eight bidesmosidic saponins (**3**, **16a**, **16b**, **19**, **21a**, **21b**, **22a**, and **22b**) were synthesized in moderate to good overall yields starting from betulin (**1**) and betulinic acid (**2**). The syntheses were achieved by a combination of Schmidt's procedures and phase-transfer conditions using fully benzoylated trichloroacetimidate and sugar bromide donors. This SAR study suggests that the relative cytotoxicities of bidesmosidic betulin and betulinic acid saponins are strongly influenced by the nature of both the aglycone and the sugar moieties. Bidesmosides **22a** and **22b** bearing α-L-rhamnopyranosyl moieties at both C-3 and C-28 positions were highly cytotoxic. Therefore, these preliminary results indicate that bidesmosidic saponins having betulin (**1**) or betulinic acid (**2**) as the aglycone may have clinical potential as anticancer agents. The relatively high polarity of these compounds should facilitate the preparation of nontoxic injectable formulations for further *in vivo* studies on animal models. Work on the evaluation of the hemolytic activity and the mechanism of action of these new "lead" compounds (**22a** and **22b**) is currently in progress in our laboratory, and results will be reported in due course.

Experimental Section

General Experimental Procedures. Chemical reagents were purchased from Sigma-Aldrich Co. Canada or Alfa Aesar Co. and were used as received. Solvents were obtained from VWR International Co. and were used as received. Air- and water-sensitive reactions were performed in flame-dried glassware under argon. Moisture-sensitive reagents were introduced via a dry syringe. Dichloromethane and acetone were distilled from anhydrous CaH_2 under argon. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. MeOH was distilled from Mg and I_2 under argon. Analytical thin-layer chromatography was performed with silica gel 60 F_{254}, 0.25 mm precoated TLC plates (Silicycle, Québec, Canada). Compounds were visualized using UV_{254} and cerium molybdate (2 g $Ce(SO_4)_4(NH_4)_4$, 5 g $MoO_4(NH_4)_2$, 200 mL H_2O, 20 mL H_2SO_4) with charring. Flash column chromatography was carried out using 230−400 mesh silica gel (Silicycle, Québec, Canada). All chemical yields represent the highest result obtained for at least three independent experiments. NMR spectra were recorded on a Bruker Avance spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C), equipped with a 5 mm QNP probe. Elucidations of chemical structures were based on ^{1}H, ^{13}C, COSY, TOCSY, HMBC, HSQC, and DEPT-135 experiments. Chemical shifts are reported in ppm (*δ*) relative to tetramethylsilane (TMS). Optical rotations were obtained at the sodium D line at ambient temperature on a Rudolph Research Analytical Autopol IV automatic polarimeter. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained at the Department of Chemistry, Université de Montréal, Québec, Canada. Compound **11**[54] was synthesized from D-glucose. Betulin (**1**) was extracted from the outer bark of *Betula papyrifera* March. and recrystallized with an azeotropic mixture of 2-butanol/H_2O (37:13) to afford crude **1** with purity >95% according to GC-MS. Betulinic acid (**2**) was purchased from Indofine Chemical Company

Scheme 5. Synthesis of Bidesmosidic Saponins (**21a**, **21b**, **22a**, and **22b**) by Schmidt's "Inverse Procedure"

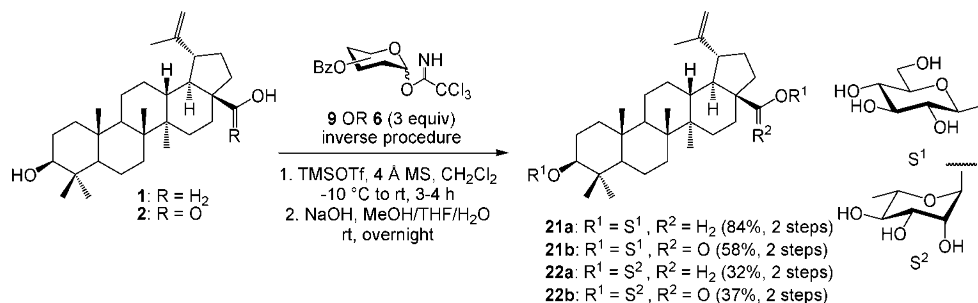
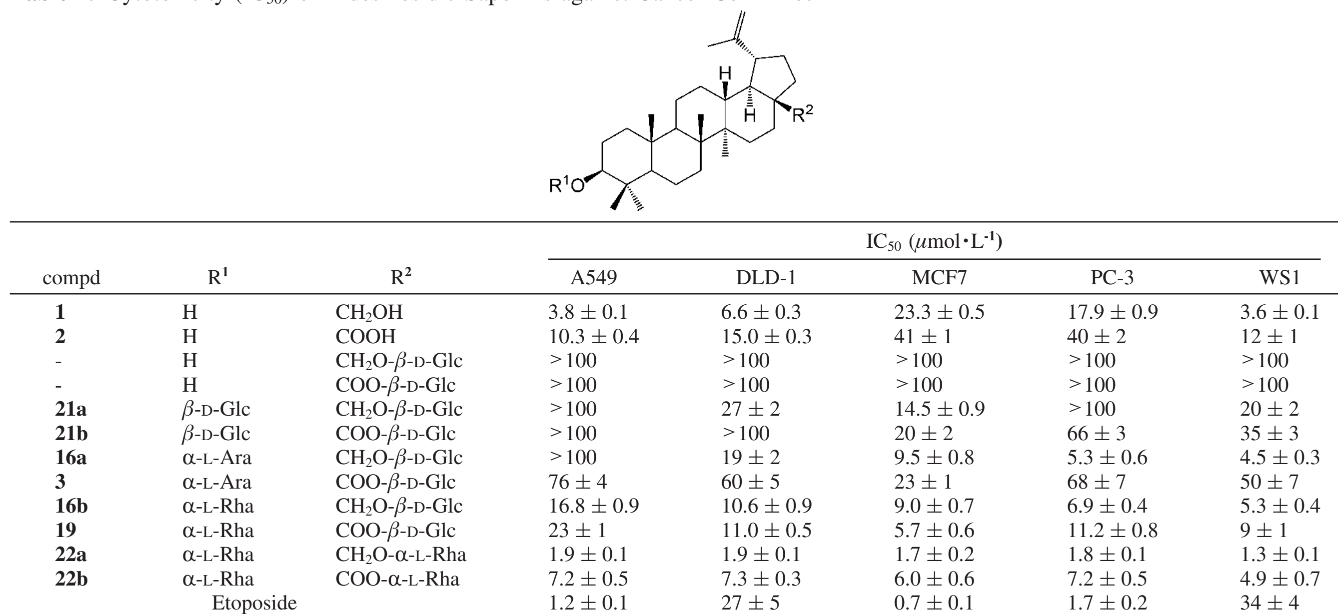


Table 2. Cytotoxicity (IC_{50}) of Bidesmosidic Saponins against Cancer Cell Lines[a]



compd	R¹	R²	IC₅₀ (μmol·L⁻¹) A549	DLD-1	MCF7	PC-3	WS1
1	H	CH_2OH	3.8 ± 0.1	6.6 ± 0.3	23.3 ± 0.5	17.9 ± 0.9	3.6 ± 0.1
2	H	COOH	10.3 ± 0.4	15.0 ± 0.3	41 ± 1	40 ± 2	12 ± 1
-	H	CH_2O-β-D-Glc	>100	>100	>100	>100	>100
-	H	COO-β-D-Glc	>100	>100	>100	>100	>100
21a	β-D-Glc	CH_2O-β-D-Glc	>100	27 ± 2	14.5 ± 0.9	>100	20 ± 2
21b	β-D-Glc	COO-β-D-Glc	>100	>100	20 ± 2	66 ± 3	35 ± 3
16a	α-L-Ara	CH_2O-β-D-Glc	>100	19 ± 2	9.5 ± 0.8	5.3 ± 0.6	4.5 ± 0.3
3	α-L-Ara	COO-β-D-Glc	76 ± 4	60 ± 5	23 ± 1	68 ± 7	50 ± 7
16b	α-L-Rha	CH_2O-β-D-Glc	16.8 ± 0.9	10.6 ± 0.9	9.0 ± 0.7	6.9 ± 0.4	5.3 ± 0.4
19	α-L-Rha	COO-β-D-Glc	23 ± 1	11.0 ± 0.5	5.7 ± 0.6	11.2 ± 0.8	9 ± 1
22a	α-L-Rha	CH_2O-α-L-Rha	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.2	1.8 ± 0.1	1.3 ± 0.1
22b	α-L-Rha	COO-α-L-Rha	7.2 ± 0.5	7.3 ± 0.3	6.0 ± 0.6	7.2 ± 0.5	4.9 ± 0.7
	Etoposide		1.2 ± 0.1	27 ± 5	0.7 ± 0.1	1.7 ± 0.2	34 ± 4

[a] Glc, glucopyranose; Rha, rhamnopyranose; Ara, arabinopyranose.

Inc. 28-*O*-β-D-Glucopyranosylbetulin,[15] 28-*O*-β-D-glucopyranosylbetulinic acid,[18] 28-*O*-*tert*-butyldiphenylsilylbetulin (**4**),[36] and betulin 3-acetate (**13**)[16] were synthesized according to reported procedures.

28-*O*-*tert*-Butyldiphenylsilylbetulin 3β-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside (7a). The acceptor **4** (750 mg, 1.10 mmol) and the donor **5** (1.00 g, 1.65 mmol) were stirred at room temperature in anhydrous CH_2Cl_2 (16.5 mL, 15 mL·mmol^{-1}) with 4 Å molecular sieves under argon during 60 min. Then, the promoter TMSOTf (12 μL, 0.055 mmol) was injected in the medium via a dry syringe while keeping rigorous anhydrous conditions. The mixture was stirred 2.5 h at room temperature and quenched by addition of Et_3N (0.61 mL, 4.4 mmol). The solvents were evaporated under reduced pressure, then the resulting oily residue was purified by flash chromatography (hexanes/Et_2O, 9:1 to 17:3) to afford **7a** (874 mg, 71%) as a white, crystalline powder: $[\alpha]^{25}_D$ +71.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.08−7.27 (25H, H-Ar), 5.78 (1H, dd, *J* = 8.7, 6.5 Hz, H-2′), 5.68 (1H, m, H-4′), 5.60 (1H, dd, *J* = 8.9, 3.5 Hz, H-3′), 4.78 (1H, d, *J* = 6.4 Hz, H-1′), 4.59 (1H, br s, H-29), 4.52 (1H, br s, H-29), 4.32 (1H, dd, *J* = 13.0, 3.8 Hz, H-5′), 3.86 (1H, dd, *J* = 12.9, 1.8 Hz, H-5′), 3.68 (1H, d, *J* = 9.9 Hz, H-28), 3.32 (1H, d, *J* = 10.0 Hz, H-28), 3.13 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 2.26 (1H, td, *J* = 11.0, 5.6 Hz, H-19), 1.64 (3H, s, H-30), 1.06 (9H, s, $C(CH_3)_3$), 0.91 (3H, s, H-27), 0.77 (3H, s, H-23), 0.75 (3H, s, H-25), 0.68 (3H, s, H-26), 0.64 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8−165.2 (3 × CO), 150.7 (C-20), 135.7−127.6 (C-Ar), 109.4 (C-29), 103.0 (C-1′), 90.1 (C-3), 70.8 (C-3′), 70.2 (C-2′), 68.7 (C-4′), 62.6 (C-5′), 61.0 (C-28), 55.5 (C-5), 50.3 (C-9), 48.4 (C-18), 48.4 (C-17), 47.8 (C-19), 42.6 (C-14), 40.7 (C-8), 39.0 (C-4), 38.6 (C-1), 37.2 (C-13), 36.8 (C-10), 34.5 (C-22), 34.1 (C-7), 29.8 (C-21), 29.5 (C-16), 27.7 (C-23), 27.0 (C-15), 26.9 ($C(CH_3)_3$), 26.1 (C-2), 25.1 (C-12), 20.7 (C-11), 19.4 ($C(CH_3)_3$), 19.1 (C-30), 18.1 (C-6), 16.0 (C-24), 16.0 (C-25), 15.7 (C-26), 14.6 (C-27); HRESIMS *m/z* 1147.6111 [M + Na]$^+$ (calcd for $C_{72}H_{88}O_9SiNa$, 1147.6090).

28-*O*-*tert*-Butyldiphenylsilylbetulin 3β-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (7b). This compound was prepared from the acceptor **4** (500 mg, 0.734 mmol) and the donor **6** (684 mg, 1.10 mmol) in the same manner as that described for compound **7a**. Purification by flash chromatography (isocratic hexanes/Et_2O, 9:1) gave **7b** (634 mg, 76%) as a white, crystalline powder: $[\alpha]^{25}_D$ +46.6 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.13−7.21 (25H, H-Ar), 5.84 (1H, dd, *J* = 10.1, 3.3 Hz, H-3′), 5.68 (1H, m, H-4′), 5.65 (1H, m, H-2′), 5.08 (1H, d, *J* = 1.1 Hz, H-1′), 4.60 (1H, d, *J* = 1.8 Hz, H-29), 4.53 (1H, br s, H-29), 4.30 (1H, m, H-5′), 3.70 (1H, d, *J* = 9.9 Hz, H-28), 3.34 (1H, d, *J* = 9.9 Hz, H-28), 3.20 (1H, t, *J* = 8.3 Hz, H-3), 2.27 (1H, td, *J* = 10.8, 5.6 Hz, H-19), 1.65 (3H, s, H-30), 1.33 (3H, d, *J* = 6.2 Hz, H-6′), 1.07 (9H, s, $C(CH_3)_3$), 1.06 (3H, s, H-23), 0.94 (3H, s, H-24), 0.94 (3H, s, H-27), 0.83 (3H, s, H-25), 0.72 (3H, s, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8−165.6 (3 × CO), 150.8 (C-20), 135.7−127.6 (C-Ar), 109.4 (C-29), 99.7 (C-1′), 90.0 (C-3), 72.0 (C-4′), 71.2 (C-2′), 70.2 (C-3′), 66.8 (C-5′), 61.1 (C-28), 55.4 (C-5), 50.3 (C-9), 48.4 (C-18), 48.4 (C-17), 47.8 (C-19), 42.6 (C-14), 40.8 (C-8), 39.1 (C-4), 38.6 (C-1), 37.2 (C-13), 36.9 (C-10), 34.5 (C-22), 34.1 (C-7), 29.9 (C-21), 29.5 (C-16), 28.3 (C-23), 27.0 (C-15), 26.9 ($C(CH_3)_3$), 25.6 (C-2), 25.1 (C-12), 20.8 (C-11), 19.4 ($C(CH_3)_3$), 19.1 (C-30), 18.3 (C-6), 17.6 (C-6′), 16.4 (C-24), 16.1 (C-25), 15.7 (C-27), 14.7 (C-26); HRESIMS *m/z* 1161.6262 [M + Na]$^+$ (calcd for $C_{73}H_{90}O_9SiNa$, 1161.6246).

Betulin 3β-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside (8a). To a solution of **7a** (200 mg, 0.178 mmol) in anhydrous THF (1.94 mL) were added HOAc (224 μL, 3.91 mmol) and 1 M TBAF in THF (3.88 mL) at room temperature under argon. The mixture was refluxed overnight or until TLC showed no remaining **7a**. The mixture was diluted with EtOAc, washed with H_2O, dried over anhydrous $MgSO_4$, and filtered, and the solvents were evaporated under reduced pressure. The residue was purified by flash chromatography (hexanes/Et_2O, 9:1 to 3:2) to furnish **8a** (117 mg, 75%): white, amorphous solid; $[\alpha]^{25}_D$ +103.6 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.09−7.27 (15H,

H-Ar), 5.77 (1H, dd, J = 8.9, 6.5 Hz, H-2′), 5.67 (1H, m, H-4′), 5.60 (1H, dd, J = 8.9, 3.5 Hz, H-3′), 4.78 (1H, d, J = 6.5 Hz, H-1′), 4.68 (1H, d, J = 1.8 Hz, H-29), 4.57 (1H, br s, H-29), 4.33 (1H, dd, J = 13.0, 3.8 Hz, H-5′), 3.88 (1H, dd, J = 12.9, 1.9 Hz, H-5′), 3.78 (1H, d, J = 10.7 Hz, H-28), 3.32 (1H, d, J = 10.7 Hz, H-28), 3.14 (1H, dd, J = 11.3, 4.8 Hz, H-3), 2.38 (1H, td, J = 10.7, 5.6 Hz, H-19), 1.68 (3H, s, H-30), 0.98 (3H, s, H-26), 0.95 (3H, s, H-27), 0.80 (3H, s, H-25), 0.76 (3H, s, H-23), 0.64 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8−165.2 (3 × CO), 150.4 (C-20), 133.3−128.3 (C-Ar), 109.7 (C-29), 103.0 (C-1′), 90.1 (C-3), 70.7 (C-3′), 70.2 (C-2′), 68.7 (C-4′), 62.6 (C-5′), 60.4 (C-28), 55.5 (C-5), 50.3 (C-9), 48.7 (C-18), 47.7 (C-17), 47.7 (C-19), 42.6 (C-14), 40.9 (C-8), 39.0 (C-4), 38.7 (C-1), 37.2 (C-13), 36.8 (C-10), 34.1 (C-7), 33.9 (C-22), 29.7 (C-21), 29.1 (C-16), 27.7 (C-23), 27.0 (C-15), 26.1 (C-2), 25.2 (C-12), 20.8 (C-11), 19.1 (C-30), 18.1 (C-6), 16.0 (C-25), 16.0 (C-24), 15.9 (C-26), 14.7 (C-27).; HRESIMS m/z 909.4957 [M + Na]$^+$ (calcd for $C_{56}H_{70}O_9Na$, 909.4912).

Betulin 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (10b). This compound was prepared from **7b** (200 mg, 0.176 mmol) in the same manner as that described for compound **8a**. Purification by flash chromatography (hexanes/EtOAc, 9:1 to 3:2) gave **10b** (138 mg, 87%): white, crystalline powder; $[\alpha]^{25}_{D}$ +76.6 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13−7.23 (15H, H−Ar), 5.82 (1H, dd, J = 10.2, 3.3 Hz, H-3′), 5.67 (1H, t, J = 10.0 Hz, H-4′), 5.64 (1H, dd, J = 3.3, 1.8 Hz, H-2′), 5.08 (1H, d, J = 1.4 Hz, H-1′), 4.69 (1H, d, J = 2.1 Hz, H-29), 4.58 (1H, br s, H-29), 4.30 (1H, ddt, J = 9.7, 6.2, 6.2 Hz, H-5′), 3.81 (1H, d, J = 10.8 Hz, H-28), 3.34 (1H, d, J = 10.8 Hz, H-28), 3.20 (1H, dd, J = 8.7, 7.5 Hz, H-3), 2.39 (1H, td, J = 10.5, 5.6 Hz, H-19), 1.68 (3H, s, H-30), 1.33 (3H, d, J = 6.2 Hz, H-6′), 1.05 (3H, s, H-23), 1.04 (3H, s, H-26), 0.98 (3H, s, H-27), 0.93 (3H, s, H-24), 0.89 (3H, s, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.9−165.6 (3 × CO), 150.5 (C-20), 133.4−128.3 (C-Ar), 109.7 (C-29), 99.7 (C-1′), 90.0 (C-3), 72.0 (C-4′), 71.2 (C-2′), 70.2 (C-3′), 66.8 (C-5′), 60.6 (C-28), 55.5 (C-5), 50.4 (C-9), 48.8 (C-18), 47.8 (C-19), 47.8 (C-17), 42.7 (C-14), 41.0 (C-8), 39.2 (C-4), 38.7 (C-1), 37.3 (C-13), 36.9 (C-10), 34.2 (C-7), 34.0 (C-22), 29.8 (C-21), 29.2 (C-16), 28.3 (C-23), 27.0 (C-15), 25.7 (C-2), 25.2 (C-12), 20.9 (C-11), 19.1 (C-30), 18.3 (C-6), 17.6 (C-6′), 16.4 (C-24), 16.2 (C-25), 16.0 (C-26), 14.8 (C-27); HRESIMS m/z 923.5111 [M + Na]$^+$ (calcd for $C_{57}H_{72}O_9Na$, 923.5069).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulin 3-acetate (14). This compound was prepared from the acceptor **13** (700 mg, 1.44 mmol) and the donor **9** (1.61 g, 2.17 mmol) in the same manner as that described for compound **7a** except for the molar volume of CH_2Cl_2 (40 mL·mmol^{-1}). Purification by flash chromatography (hexanes/EtOAc, 4:1 to 7:3) gave **14** (903 mg, 60%): white foam; $[\alpha]^{25}_{D}$ +24.7 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06−7.26 (20H, H-Ar), 5.93 (1H, t, J = 9.7 Hz, H-3″), 5.67 (1H, t, J = 9.7 Hz, H-4″), 5.56 (1H, dd, J = 9.8, 8.0 Hz, H-2″), 4.79 (1H, d, J = 8.0 Hz, H-1″), 4.65 (1H, m, H-6″), 4.63 (1H, m, H-29), 4.55 (1H, m, H-29), 4.53 (1H, m, H-6″), 4.45 (1H, m, H-3), 4.17 (1H, ddd, J = 9.4, 5.6, 3.3 Hz, H-5″), 3.67 (1H, d, J = 8.9 Hz, H-28), 3.58 (1H, d, J = 8.9 Hz, H-28), 2.28 (1H, m, H-19), 2.05 (3H, s, CH_3CO), 1.63 (3H, s, H-30), 0.84 (3H, s, H-23), 0.84 (3H, s, H-24), 0.83 (3H, s, H-26), 0.82 (3H, s, H-27), 0.80 (3H, s, H-25); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (CH_3*C*O), 166.2−165.3 (4 × CO), 150.4 (C-20), 133.5−128.3 (C-Ar), 109.7 (C-29), 102.1 (C-1″), 80.9 (C-3), 72.9 (C-3″), 72.2 (C-5″), 71.8 (C-2″), 70.1 (C-4″), 68.9 (C-28), 63.3 (C-6″), 55.3 (C-5), 50.2 (C-9), 48.6 (C-18), 48.0 (C-19), 47.0 (C-17), 42.5 (C-14), 40.7 (C-8), 38.3 (C-1), 37.8 (C-4), 37.6 (C-13), 37.0 (C-10), 34.7 (C-22), 33.8 (C-7), 29.6 (C-21), 29.2 (C-16), 28.0 (C-23), 27.0 (C-15), 25.0 (C-12), 23.7 (C-2), 21.4 (*C*H_3CO), 20.8 (C-11), 19.0 (C-30), 18.1 (C-6), 16.5 (C-24), 16.2 (C-25), 15.8 (C-26), 14.7 (C-27); HRESIMS m/z 1085.5384 [M + Na]$^+$ (calcd for $C_{66}H_{78}O_{12}Na$, 1085.5386).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulin (15). To a solution of **14** (840 mg, 0.790 mmol) in anhydrous CH_2Cl_2/MeOH (1:2) (60 mL) was added AcCl (1.19 mL, 16.8 mmol) at 0 °C (ice/water bath). The mixture was stirred overnight at room temprature or until TLC (hexanes/EtOAc, 7:3) showed no remaining **14**. Then, the reaction was quenched with Et_3N (4.68 mL, 33.6 mmol) and the solvents were evaporated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 4:1 to 3:2) to afford **15** (523 mg, 75%, corrected yield) as a white crystalline powder along with **14** (87 mg, 10%, recovery yield) as a white foam: $[\alpha]^{25}_{D}$ +27.0 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05−7.25 (20H, H-Ar), 5.93 (1H, t, J = 9.7 Hz, H-3″), 5.67 (1H, t, J = 9.7 Hz, H-4″), 5.56 (1H, dd, J = 9.7, 7.8 Hz, H-2″), 4.79 (1H, d, J = 8.0 Hz, H-1″), 4.64 (1H, m, H-6″), 4.63 (1H, m, H-29), 4.54 (1H, m, H-29), 4.53 (1H, m, H-6″), 4.17 (1H, m, H-5″), 3.66 (1H, d, J = 8.9 Hz, H-28), 3.58 (1H, d, J = 8.9 Hz, H-28), 3.17 (1H, dd, J = 11.0, 4.6 Hz, H-3), 2.27 (1H, m, H-19), 1.63 (3H, s, H-30), 0.96 (3H, s, H-23), 0.83 (3H, s, H-26), 0.83 (3H, s, H-27), 0.77 (3H, s, H-25), 0.76 (3H, s, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1−165.0 (4 × CO), 150.4 (C-20), 133.4−128.3 (C-Ar), 109.6 (C-29), 102.0 (C-1″), 78.9 (C-3), 72.8 (C-3″), 72.2 (C-5″), 71.7 (C-2″), 70.0 (C-4″), 68.8 (C-28), 63.3 (C-6″), 55.2 (C-5), 50.3 (C-9), 48.6 (C-18), 48.0 (C-19), 46.9 (C-17), 42.5 (C-14), 40.7 (C-8), 38.8 (C-4), 38.6 (C-1), 37.6 (C-13), 37.1 (C-10), 34.7 (C-22), 33.8 (C-7), 29.6 (C-21), 29.2 (C-16), 28.0 (C-23), 27.3 (C-2), 27.0 (C-15), 25.0 (C-12), 20.8 (C-11), 19.0 (C-30), 18.1 (C-6), 16.1 (C-25), 15.7 (C-26), 15.4 (C-24), 14.8 (C-27); HRESIMS m/z 1043.5295 [M + Na]$^+$ (calcd for $C_{64}H_{76}O_{11}Na$, 1043.5280).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulin 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside (10a). The acceptor **15** (150 mg, 0.147 mmol) and the donor **5** (134 mg, 0.220 mmol) were stirred at room temperature in anhydrous CH_2Cl_2 (2.9 mL) with 4 Å molecular sieves under argon during 60 min. The temperature was lowered to 0 °C with an ice/water bath, then a solution of TMSOTf in CH_2Cl_2 (100 μL, 150 mM) was injected in the medium via a dry syringe while keeping rigorous anhydrous conditions. The mixture was stirred 3 h at room temperature and quenched by addition of Et_3N (82 μL, 0.59 mmol). The solvents were evaporated under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc, 9:1 to 3:2) to afford **10a** (132 mg, 62%) as a white foam: $[\alpha]^{25}_{D}$ +80.3 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10−7.25 (35H, H-Ar), 5.94 (1H, t, J = 9.7 Hz, H-3″), 5.78 (1H, dd, J = 8.9, 6.7 Hz, H-2′), 5.68 (1H, m, H-4′), 5.67 (1H, m, H-4″), 5.60 (1H, dd, J = 9.1, 3.5 Hz, H-3′), 5.56 (1H, dd, J = 9.9, 8.0 Hz, H-2″), 4.79 (1H, m, H-1″), 4.78 (1H, m, H-1′), 4.65 (1H, m, H-6″), 4.63 (1H, br s, H-29), 4.55 (1H, br s, H-29), 4.53 (1H, m, H-6″), 4.33 (1H, dd, J = 13.0, 3.5 Hz, H-5′), 4.17 (1H, ddd, J = 9.5, 5.4, 3.3 Hz, H-5″), 3.87 (1H, dd, J = 13.0, 1.9 Hz, H-5′), 3.66 (1H, d, J = 8.8 Hz, H-28), 3.57 (1H, d, J = 8.8 Hz, H-28), 3.12 (1H, dd, J = 11.3, 4.6 Hz, H-3), 2.28 (1H, m, H-19), 1.62 (3H, s, H-30), 0.81 (3H, s, H-27), 0.79 (3H, s, H-26), 0.76 (3H, s, H-23), 0.76 (3H, s, H-25), 0.65 (3H, s, H-24), 0.58 (1H, d, J = 10.8 Hz, H-5), 0.50 (1H, br d, J = 13.5 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1−164.9 (7 × CO), 150.3 (C-20), 133.4−128.3 (C-Ar), 109.6 (C-29), 103.0 (C-1′), 102.0 (C-1″), 90.0 (C-3), 72.8 (C-3″), 72.1 (C-5″), 71.7 (C-2″), 70.7 (C-3′), 70.2 (C-2′), 70.0 (C-4″), 68.9 (C-28), 68.7 (C-4′), 63.2 (C-6″), 62.6 (C-5′), 55.4 (C-5), 50.2 (C-9), 48.5 (C-18), 47.9 (C-19), 46.9 (C-17), 42.4 (C-14), 40.6 (C-8), 38.9 (C-4), 38.6 (C-1), 37.6 (C-13), 36.7 (C-10), 34.6 (C-22), 33.7 (C-7), 29.6 (C-21), 29.1 (C-16), 27.6 (C-23), 26.9 (C-15), 26.0 (C-2), 25.0 (C-12), 20.7 (C-11), 19.0 (C-30), 17.9 (C-6), 16.0 (C-24), 16.0 (C-25), 15.7 (C-26), 14.7 (C-27); HRESIMS m/z 1487.6499 [M + Na]$^+$ (calcd for $C_{90}H_{96}O_{18}Na$, 1487.6489).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulin 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (10b). This compound was prepared from the acceptor **15** (17 mg, 0.017 mmol) and the donor **6** (16 mg, 0.025 mmol) in the same manner as that described for compound **10a** except for the concentration of the solution of TMSOTf in CH_2Cl_2 (20 mM). Purification by flash chromatography (hexanes/EtOAc, 9:1 to 3:1) gave **10b** (18 mg, 72%) as a white foam: $[\alpha]^{25}_{D}$ +57.1 (c 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15−7.24 (35H, H-Ar), 5.95 (1H, t, J = 9.7 Hz, H-3″), 5.83 (1H, dd, J = 10.2, 3.3 Hz, H-3′), 5.68 (1H, m, H-4″), 5.68 (1H, m, H-4′), 5.65 (1H, m, H-2′), 5.56 (1H, dd, J = 9.9, 8.0 Hz, H-2″), 5.07 (1H, d, J = 1.3 Hz, H-1′), 4.80 (1H, d, J = 8.1 Hz, H-1″), 4.66 (1H, m, H-6″), 4.63 (1H, m, H-29), 4.55 (1H, m, H-29), 4.54 (1H, m, H-6″), 4.32 (1H, dd, J = 9.7, 6.0 Hz, H-5′), 4.18 (1H, ddd, J = 9.4, 5.4, 3.3 Hz, H-5″), 3.67 (1H, d, J = 9.1 Hz, H-28), 3.59 (1H, d, J = 9.1 Hz, H-28), 3.18 (1H, t, J = 8.1 Hz, H-3), 2.29 (1H, m, H-19), 1.63 (3H, s, H-30), 1.33 (3H, d, J = 6.2 Hz, H-6′), 1.04 (3H, s, H-23), 0.94 (3H, s, H-24), 0.85 (3H, s, H-26), 0.84 (3H, s, H-25), 0.83 (3H, s, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.2−165.0 (7 × CO), 150.4 (C-20), 133.4−128.3 (C-Ar), 109.6 (C-29), 102.1 (C-1″), 99.7 (C-1′), 90.0 (C-3), 72.8 (C-3″), 72.2 (C-5″), 72.0 (C-4″), 71.7 (C-2″), 71.2 (C-2′), 70.2 (C-3′), 70.0 (C-4′), 68.9 (C-28), 66.8 (C-5′), 63.3 (C-6″), 55.4 (C-5), 50.2 (C-9), 48.6 (C-18), 48.0 (C-19), 46.9 (C-17), 42.5 (C-14), 40.7 (C-8), 39.1 (C-4), 38.6 (C-1), 37.6 (C-13), 36.8 (C-10), 34.7 (C-22), 33.8 (C-7), 29.6 (C-21),

Table 3. ^{13}C NMR Data of Bidesmosidic Saponins **3**, **16a**, **16b**, **19**, **21a**, **21b**, **22a**, and **22b**[a]

position	3[b]	16a[b]	16b[b]	19[c]	21a[d]	21b[c]	22a[b]	22b[c]
1	39.5 (t)	39.2 (t)	39.1 (t)	39.9 (t)	39.4 (t)	40.1 (t)	39.0 (t)	39.9 (t)
2	26.7 (t)	26.4 (t)	26.0 (t)	26.8 (t)	27.1 (t)	27.2 (t)	25.9 (t)	26.8 (t)
3	90.3 (d)	90.2 (d)	89.7 (d)	90.4 (d)	89.2 (d)	90.9 (d)	89.7 (d)	90.4 (d)
4	39.8 (s)	39.6 (s)	39.5 (s)	40.2 (s)	40.0 (s)	40.3 (s)	39.4 (s)	40.2 (s)
5	56.5 (d)	56.1 (d)	55.9 (d)	56.9 (d)	56.2 (d)	57.2 (d)	55.8 (d)	56.9 (d)
6	18.8 (t)	18.6 (t)	18.7 (t)	19.4 (t)	18.8 (t)	19.3 (t)	18.6 (t)	19.4 (t)
7	35.0 (t)	34.6 (t)	34.6 (t)	35.5 (t)	34.8 (t)	35.5 (t)	34.6 (t)	35.6 (t)
8	41.5 (s)	41.4 (s)	41.4 (s)	42.0 (s)	41.5 (s)	42.1 (s)	41.3 (s)	42.0 (s)
9	51.4 (d)	50.8 (d)	50.9 (d)	52.0 (d)	51.0 (d)	52.0 (d)	50.8 (d)	51.9 (d)
10	37.6 (s)	37.3 (s)	37.3 (s)	38.1 (s)	37.4 (s)	38.1 (s)	37.2 (s)	38.1 (s)
11	21.6 (t)	21.3 (t)	21.3 (t)	22.1 (t)	21.3 (t)	22.1 (t)	21.2 (t)	22.2 (t)
12	26.3 (t)	25.7 (t)	25.7 (t)	26.9 (t)	26.0 (t)	26.9 (t)	25.6 (t)	26.9 (t)
13	38.8 (d)	38.0 (d)	38.0 (d)	39.4 (d)	38.0 (d)	39.4 (d)	38.0 (d)	40.0 (d)
14	43.1 (s)	43.1 (s)	43.1 (s)	43.6 (s)	43.3 (s)	43.6 (s)	43.1 (s)	43.7 (s)
15	30.2 (t)	27.5 (t)	27.5 (t)	30.8 (t)	28.0 (t)	30.9 (t)	27.5 (t)	30.8 (t)
16	32.4 (t)	29.9 (t)	29.9 (t)	32.8 (t)	30.4 (t)	32.8 (t)	30.1 (t)	33.1 (t)
17	57.4 (s)	47.6 (s)	47.6 (s)	57.9 (s)	48.1 (s)	58.0 (s)	47.3 (s)	58.3 (s)
18	50.1 (d)	49.3 (d)	49.3 (d)	50.6 (d)	49.5 (d)	50.6 (d)	49.2 (d)	50.5 (d)
19	47.7 (d)	48.3 (d)	48.3 (d)	48.4 (d)	48.4 (d)	48.4 (d)	48.3 (d)	48.8 (d)
20	151.1 (s)	151.0 (s)	151.0 (s)	151.8 (s)	151.3 (s)	151.9 (s)	150.8 (s)	151.5 (s)
21	31.0 (t)	30.1 (t)	30.1 (t)	31.5 (t)	30.5 (t)	31.5 (t)	30.3 (t)	31.8 (t)
22	37.1 (t)	35.1 (t)	35.1 (t)	37.5 (t)	35.6 (t)	37.5 (t)	35.3 (t)	38.0 (t)
23	28.2 (q)	28.2 (q)	28.3 (q)	28.7 (q)	28.5 (q)	28.4 (q)	28.3 (q)	28.7 (q)
24	16.5 (q)	16.5 (q)	16.4 (q)	16.8 (q)	16.4 (q)	16.8 (q)	16.4 (q)	16.8 (q)
25	16.6 (q)	16.5 (q)	16.4 (q)	16.8 (q)	17.2 (q)	16.8 (q)	16.4 (q)	16.8 (q)
26	16.3 (q)	16.4 (q)	16.3 (q)	16.7 (q)	16.7 (q)	16.7 (q)	16.2 (q)	16.8 (q)
27	15.1 (q)	15.1 (q)	15.1 (q)	15.2 (q)	15.3 (q)	15.2 (q)	15.0 (q)	15.2 (q)
28	175.9 (s)	68.9 (t)	68.8 (t)	176.1 (s)	68.9 (t)	176.2 (s)	66.4 (t)	175.6 (s)
29	110.1 (t)	110.0 (t)	109.9 (t)	110.3 (t)	110.4 (t)	110.3 (t)	110.0 (t)	110.6 (t)
30	19.5 (q)	19.4 (q)	19.3 (q)	19.5 (q)	19.6 (q)	19.5 (q)	19.3 (q)	19.6 (q)
1′	106.2 (d)	105.5 (d)	103.3 (d)	104.4 (d)	107.3 (d)	106.8 (d)	103.1 (d)	104.4 (d)
2′	72.1 (d)	71.7 (d)	71.5 (d)	72.5 (d)	76.2 (d)	75.7 (d)	71.4 (d)	72.5 (d)
3′	73.6 (d)	73.1 (d)	71.9 (d)	72.5 (d)	79.2 (d)	78.3 (d)	71.9 (d)	72.6 (d)
4′	68.4 (d)	67.8 (d)	73.4 (d)	74.1 (d)	72.2 (d)	71.7 (d)	73.3 (d)	74.1 (d)
5′	65.4 (t)	64.9 (t)	68.8 (d)	69.9 (d)	78.7 (d)	77.7 (d)	68.8 (d)	69.9 (d)
6′			17.5 (q)	17.9 (q)	63.4 (t)	62.8 (t)	17.7 (q)	17.9 (q)
1″	94.6 (d)	104.3 (d)	104.4 (d)	95.2 (d)	106.4 (d)	95.2 (d)	101.1 (d)	95.1 (d)
2″	73.4 (d)	74.2 (d)	74.2 (d)	74.1 (d)	75.8 (d)	74.1 (d)	71.3 (d)	71.4 (d)
3″	77.7 (d)	76.9 (d)	77.0 (d)	78.4 (d)	79.0 (d)	78.4 (d)	71.8 (d)	72.8 (d)
4″	70.6 (d)	70.8 (d)	70.8 (d)	71.1 (d)	72.2 (d)	71.1 (d)	73.1 (d)	73.4 (d)
5″	78.0 (d)	76.3 (d)	76.5 (d)	78.8 (d)	79.0 (d)	78.8 (d)	68.7 (d)	69.9 (d)
6″	62.0 (t)	62.3 (t)	62.1 (t)	62.4 (t)	63.3 (t)	62.4 (t)	17.5 (q)	18.2 (q)

[a] Spectra recorded at 100 MHz. The multiplicities were deduced from DEPT experiments. [b] $CDCl_3/CD_3OD$. [c] CD_3OD. [d] Pyridine-d_5.

29.2 (C-16), 28.2 (C-23), 26.9 (C-15), 25.6 (C-2), 25.0 (C-12), 20.8 (C-11), 19.0 (C-30), 18.1 (C-6), 17.6 (C-6′), 16.4 (C-24), 16.1 (C-25), 15.7 (C-26), 14.8 (C-27); HRESIMS *m/z* 1501.6648 [M + Na]$^+$ (calcd for $C_{91}H_{98}O_{18}Na$, 1501.6645).

28-*O*-*β*-D-Glucopyranosylbetulin 3*β*-*O*-α-L-arabinopyranoside (16a). To a solution of **10a** (94 mg, 0.064 mmol) in $MeOH/THF/H_2O$ (1:2:1) (4.4 mL) was added NaOH (52 mg, 1.3 mmol). The reaction mixture was stirred 5 h at room temperature or until TLC (CH_2Cl_2/MeOH, 9:1) showed no remaining **10a** and then acidified to pH ≈ 4 with aqueous HCl 10%. The solvents were evaporated under reduced pressure. The residue was purified by C-18 reversed-phase flash chromatography ($MeOH/H_2O$, 4:1 to 9:1) to furnish **16a** (40 mg, 86%) as a white, amorphous powder: $[\alpha]^{25}_D$ −15.6 (*c* 0.1, MeOH); ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1, 400 MHz) *δ* 4.68 (1H, d, *J* = 1.6 Hz, H-29), 4.58 (1H, br s, H-29), 4.34 (1H, d, *J* = 5.9 Hz, H-1′), 4.25 (1H, d, *J* = 7.8 Hz, H-1″), 3.89 (1H, m, H-6″), 3.88 (1H, m, H-4′), 3.88 (1H, m, H-5′), 3.79 (1H, dd, *J* = 11.9, 4.6 Hz, H-6″), 3.68 (1H, m, H-28), 3.65 (1H, m, H-2′), 3.61 (1H, m, H-3′), 3.61 (1H, m, H-28), 3.53 (1H, dd, *J* = 13.8, 3.8 Hz, H-5′), 3.45 (1H, m, H-4″), 3.44 (1H, m, H-3″), 3.31 (1H, m, H-5″), 3.27 (1H, m, H-2″), 3.13 (1H, dd, *J* = 11.3, 4.3 Hz, H-3), 2.43 (1H, td, *J* = 10.3, 5.7 Hz, H-19), 1.69 (3H, s, H-30), 1.04 (3H, s, H-26), 1.01 (3H, s, H-23), 0.98 (3H, s, H-27), 0.84 (3H, s, H-25), 0.82 (3H, s, H-24), 0.73 (1H, d, *J* = 10.3 Hz, H-5); ^{13}C NMR, see Table 3; HRESIMS *m/z* 759.4635 [M + Na]$^+$ (calcd for $C_{41}H_{68}O_{11}Na$, 759.4654).

28-*O*-*β*-D-Glucopyranosylbetulin 3*β*-*O*-α-L-rhamnopyranoside (16b). This compound was prepared from **10b** (84 mg, 0.057 mmol) in the same manner as that described for compound **16a**. Purification by C-18 reversed-phase flash chromatography ($MeOH/H_2O$, 4:1, to 100% MeOH) gave **16b** (33 mg, 80%): white, amorphous powder; $[\alpha]^{25}_D$ −42.8 (*c* 0.2, MeOH); ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1, 400 MHz) *δ* 4.76 (1H, br s, H-1′), 4.68 (1H, br s, H-29), 4.58 (1H, br s, H-29), 4.25 (1H, d, *J* = 7.8 Hz, H-1″), 3.90 (1H, m, H-6″), 3.89 (1H, m, H-2′), 3.78 (1H, m, H-6″), 3.75 (1H, m, H-5′), 3.70 (1H, m, H-28), 3.69 (1H, m, H-3′), 3.62 (1H, d, *J* = 9.2 Hz, H-28), 3.43 (1H, m, H-4″), 3.42 (1H, m, H-3″), 3.39 (1H, m, H-4′), 3.31 (1H, m, H-5″), 3.27 (1H, m, H-2″), 3.08 (1H, dd, *J* = 11.4, 4.6 Hz, H-3), 2.43 (1H, m, H-19), 2.09 (1H, br d, *J* = 12.1 Hz, H-16), 1.69 (3H, s, H-30), 1.27 (3H, d, *J* = 6.0 Hz, H-6′), 1.05 (3H, s, H-26), 0.99 (3H, s, H-27), 0.93 (3H, s, H-23), 0.85 (3H, s, H-25), 0.76 (3H, s, H-24); ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1, 100 MHz), see Table 3; HRESIMS *m/z* 773.4794 [M + Na]$^+$ (calcd for $C_{42}H_{70}O_{11}Na$, 773.4810).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulinic Acid (17). To a solution of the acceptor **2** (500 mg, 1.10 mmol) and the donor **12** (939 mg, 1.42 mmol) in CH_2Cl_2 (12.7 mL) were added H_2O (12.7 mL), K_2CO_3 (378 mg, 2.74 mmol), and Bu_4NBr (141 mg, 0.438 mmol). The resulting mixture was vigorously stirred and refluxed for 6 h. Then, the mixture was diluted with CH_2Cl_2 and washed with H_2O and brine. The solvents of the dried ($MgSO_4$) organic solution were evaporated under reduced pressure. The brown residue was purified by flash chromatography (100% CH_2Cl_2 to CH_2Cl_2/MeOH, 49:1) to afford **17** (1.015 g, 90%): white, crystalline powder; $[\alpha]^{25}_D$ +38.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 8.07−7.25 (20H, H-Ar), 6.03 (1H, d, *J* = 8.4 Hz, H-1″), 6.02 (1H, t, *J* = 9.5 Hz, H-3″), 5.76 (1H, dd, *J* = 9.9, 8.4 Hz, H-2″), 5.73 (1H, t, *J* = 9.8 Hz, H-4″), 4.71 (1H, br s, H-29), 4.59 (1H, m, H-6″), 4.58 (1H, m, H-29), 4.48 (1H, dd, *J* = 12.2, 5.6 Hz, H-6″), 4.29 (1H, ddd, *J* = 9.5, 5.3, 2.9 Hz, H-5″), 3.13 (1H, dd, *J* = 11.0, 4.6 Hz, H-3), 2.93 (1H, td, *J* = 11.1, 4.8 Hz, H-19), 2.17 (1H, br d, *J* = 13.2 Hz, H-16), 2.03 (1H, td, *J* = 12.2, 3.2 Hz, H-13), 1.91 (1H, dd, *J* = 12.7, 8.0 Hz, H-22), 1.63 (3H, s, H-30),

0.93 (3H, s, H-23), 0.79 (3H, s, H-27), 0.73 (3H, s, H-24), 0.68 (3H, s, H-25), 0.60 (1H, br d, J = 14.3 Hz, H-15), 0.54 (1H, br d, J = 10.5 Hz, H-5), 0.47 (3H, s, H-26), 0.38 (1H, br d, J = 11.0 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0 (C-28), 166.1−164.7 (4 × CO), 150.3 (C-20), 133.5−128.3 (C-Ar), 109.5 (C-29), 91.4 (C-1″), 78.9 (C-3), 73.0 (C-5″), 72.8 (C-3″), 70.3 (C-2″), 69.4 (C-4″), 62.7 (C-6″), 56.8 (C-17), 55.2 (C-5), 50.4 (C-9), 49.1 (C-18), 46.6 (C-19), 42.2 (C-14), 40.2 (C-8), 38.8 (C-4), 38.6 (C-1), 38.0 (C-13), 37.0 (C-10), 36.3 (C-22), 33.4 (C-7), 31.5 (C-16), 30.2 (C-21), 29.9 (C-15), 28.0 (C-23), 27.4 (C-2), 25.4 (C-12), 20.7 (C-11), 19.5 (C-30), 18.0 (C-6), 16.0 (C-25), 15.4 (C-26), 15.4 (C-24), 14.5 (C-27); HRESIMS m/z 1057.5114 [M + Na]$^+$ (calcd for $C_{64}H_{74}O_{12}Na$, 1057.5073).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulinic acid 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside (18a). This compound was prepared from the acceptor **17** (250 mg, 0.241 mmol) and the donor **5** (220 mg, 0.362 mmol) in the same manner as that described for compound **7a** except for the molar volume of CH_2Cl_2 (20 mL·mmol^{-1}). Purification by flash chromatography (hexanes/EtOAc, 9:1 to 7:3) gave **18a** (224 mg, 63%): white, crystalline powder; $[\alpha]^{25}_D$ +88.9 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09−7.23 (35H, H-Ar), 6.04 (1H, m, H-1″), 6.03 (1H, m, H-3″), 5.78 (1H, m, H-2′), 5.76 (1H, m, H-2″), 5.75 (1H, m, H-4″), 5.68 (1H, m, H-4′), 5.61 (1H, m, H-3′), 4.77 (1H, d, H-1′), 4.71 (1H, m, H-29), 4.60 (1H, m, H-6″), 4.58 (1H, m, H-29), 4.50 (1H, m, H-6″), 4.32 (1H, m, H-5′), 4.30 (1H, m, H-5″), 3.87 (1H, m, H-5′), 3.09 (1H, m, H-3), 2.94 (1H, m, H-19), 1.64 (3H, s, H-30), 0.77 (3H, s, H-27), 0.74 (3H, s, H-23), 0.67 (3H, s, H-25), 0.62 (3H, s, H-24), 0.44 (3H, s, H-26); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0 (C-28), 166.0−164.7 (7 × CO), 150.2 (C-20), 133.5−128.3 (C-Ar), 109.6 (C-29), 103.0 (C-1′), 91.4 (C-1″), 90.1 (C-3), 73.0 (C-5″), 72.8 (C-3″), 70.7 (C-3′), 70.2 (C-2″), 70.2 (C-2′), 69.4 (C-4″), 68.7 (C-4′), 62.7 (C-6″), 62.7 (C-5′), 56.8 (C-17), 55.4 (C-5), 50.3 (C-9), 49.1 (C-18), 46.6 (C-19), 42.1 (C-14), 40.2 (C-8), 38.9 (C-4), 38.6 (C-1), 38.0 (C-13), 36.7 (C-10), 36.3 (C-22), 33.3 (C-7), 31.5 (C-16), 30.2 (C-21), 29.8 (C-15), 27.7 (C-23), 26.0 (C-2), 25.4 (C-12), 20.7 (C-11), 19.5 (C-30), 17.8 (C-6), 16.1 (C-24), 15.9 (C-25), 15.3 (C-26), 14.4 (C-27); HRESIMS m/z 1501.6347 [M + Na]$^+$ (calcd for $C_{90}H_{94}O_{19}Na$, 1501.6282).

28-*O*-2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranosylbetulinic acid 3*β*-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranoside (18b). This compound was prepared from the acceptor **17** (250 mg, 0.241 mmol) and the donor **6** (225 mg, 0.362 mmol) in the same manner as that described for compound **7a** except for the molar volume of CH_2Cl_2 (20 mL·mmol^{-1}). Purification by flash chromatography (hexanes/EtOAc, 9:1 to 4:1) gave **18b** (311 mg, 86%): white, amorphous powder; $[\alpha]^{25}_D$ +72.5 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.10−7.21 (35H, H-Ar), 6.08 (1H, m, H-1″), 6.07 (1H, m, H-3″), 5.83 (1H, m, H-3′), 5.82 (1H, m, H-2″), 5.77 (1H, m, H-4″), 5.69 (1H, m, H-4′), 5.67 (1H, m, H-2′), 5.08 (1H, br s, H-1′), 4.72 (1H, br s, H-29), 4.62 (1H, dd, J = 12.3, 2.9 Hz, H-6″), 4.59 (1H, br s, H-29), 4.52 (1H, dd, J = 12.3, 5.4 Hz, H-6″), 4.34 (1H, m, H-5″), 4.33 (1H, m, H-5′), 3.17 (1H, t, J = 8.1 Hz, H-3), 2.96 (1H, td, J = 10.8, 4.6 Hz, H-19), 2.20 (1H, br d, J = 12.7 Hz, H-16), 1.64 (3H, s, H-30), 1.34 (3H, d, J = 6.2 Hz, H-6′), 1.03 (3H, s, H-23), 0.92 (3H, s, H-24), 0.81 (3H, s, H-27), 0.77 (3H, s, H-25), 0.51 (3H, s, H-26), 0.44 (1H, br d, J = 11.4 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0 (C-28), 166.0−163.5 (7 × CO), 150.1 (C-20), 133.6−128.2 (C-Ar), 109.5 (C-29), 99.7 (C-1′), 91.4 (C-1″), 90.0 (C-3), 72.9 (C-5″), 72.8 (C-3″), 71.9 (C-4′), 71.1 (C-2′), 70.2 (C-3′), 70.2 (C-2″), 69.3 (C-4″), 66.7 (C-5′), 62.7 (C-6″), 56.7 (C-17), 55.3 (C-5), 50.3 (C-9), 49.0 (C-18), 46.6 (C-19), 42.1 (C-14), 40.2 (C-8), 39.0 (C-4), 38.5 (C-1), 37.9 (C-13), 36.7 (C-10), 36.3 (C-22), 33.3 (C-7), 31.4 (C-16), 30.2 (C-21), 29.8 (C-15), 28.2 (C-23), 25.5 (C-2), 25.3 (C-12), 20.7 (C-11), 19.4 (C-30), 17.9 (C-6), 17.5 (C-6′), 16.3 (C-24), 16.0 (C-25), 15.3 (C-26), 14.4 (C-27); HRESIMS m/z 1515.6419 [M + Na]$^+$ (calcd for $C_{91}H_{96}O_{19}Na$, 1515.6438).

28-*O*-*β*-D-Glucopyranosylbetulinic acid 3*β*-*O*-α-L-arabinopyranoside (3). This compound was prepared from **18a** (100 mg, 0.068 mmol) in the same manner as that described for compound **16a**. Purification by C-18 reversed-phase flash chromatography (MeOH/H_2O, 3:2 to 9:1) gave **3** (38 mg, 75%): white, amorphous powder; $[\alpha]^{25}_D$ +12.2 (c 0.1, MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD, 1:2, 400 MHz) δ 5.51 (1H, d, J = 8.1 Hz, H-1″), 4.72 (1H, br s, H-29), 4.60 (1H, br s, H-29), 4.31 (1H, d, J = 6.3 Hz, H-1′), 3.86 (1H, m, H-5′), 3.86 (1H, m, H-6″), 3.84 (1H, m, H-4′), 3.74 (1H, dd, J = 12.1, 4.0 Hz, H-6″), 3.61 (1H, dd, J = 8.4, 6.4 Hz, H-2′), 3.55 (1H, dd, J = 8.4, 3.0 Hz, H-3′), 3.52 (1H, d, J = 10.3 Hz, H-5′), 3.46 (1H, m, H-3″), 3.42 (1H, m, H-4″), 3.41 (1H, m, H-5″), 3.37 (1H, m, H-2″), 3.13 (1H, dd, J = 11.1, 4.0 Hz, H-3), 3.00 (1H, td, J = 11.0, 4.6 Hz, H-19), 1.69 (3H, s, H-30), 1.01 (3H, s, H-23), 0.99 (3H, s, H-27), 0.95 (3H, s, H-26), 0.85 (3H, s, H-25), 0.81 (3H, s, H-24), 0.73 (1H, d, J = 9.5 Hz, H-5); ^{13}C NMR, see Table 3; HRESIMS m/z 773.4444 [M + Na]$^+$ (calcd for $C_{41}H_{66}O_{12}Na$, 773.4447).

28-*O*-*β*-D-Glucopyranosylbetulinic acid 3*β*-*O*-α-L-rhamnopyranoside (19). This compound was prepared from **18b** (147 mg, 0.0986 mmol) in the same manner as that described for compound **16a**. Purification by C-18 reversed-phase flash chromatography (MeOH/H_2O, 3:2 to 9:1) gave **19** (61 mg, 81%): white, amorphous powder; $[\alpha]^{25}_D$ −32.4 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.49 (1H, d, J = 8.1 Hz, H-1″), 4.71 (1H, m, H-1′), 4.71 (1H, m, H-29), 4.59 (1H, m, H-29), 3.84 (1H, m, H-6″), 3.82 (1H, m, H-2′), 3.70 (1H, m, H-5′), 3.70 (1H, m, H-6″), 3.63 (1H, dd, J = 9.5, 3.3 Hz, H-3′), 3.43 (1H, m, H-3″), 3.38 (1H, m, H-4″), 3.37 (1H, m, H-5″), 3.36 (1H, m, H-4′), 3.31 (1H, m, H-2″), 3.06 (1H, dd, J = 11.6, 4.8 Hz, H-3), 3.00 (1H, td, J = 10.8, 6.2 Hz, H-19), 1.69 (3H, s, H-30), 1.22 (3H, d, J = 6.2 Hz, H-6′), 1.00 (3H, s, H-27), 0.95 (3H, s, H-26), 0.93 (3H, s, H-23), 0.86 (3H, s, H-25), 0.77 (3H, s, H-24); ^{13}C NMR, see Table 3; HRESIMS m/z 787.4607 [M + Na]$^+$ (calcd for $C_{42}H_{68}O_{12}Na$, 787.4603).

28-*O*-*β*-D-Glucopyranosylbetulin 3*β*-*O*-*β*-D-glucopyranoside (21a). A solution of the acceptor **1** (250 mg, 0.565 mmol) in anhydrous CH_2Cl_2 (11.3 mL) was stirred for 60 min with 4 Å molecular sieves at −10 °C (ice water/acetone bath). TMSOTf (20 μL, 0.113 mmol) was added under argon while keeping rigorous anhydrous conditions. Then, a solution of the donor **9** (1.26 g, 1.70 mmol) in anhydrous CH_2Cl_2 (8.5 mL) was added dropwise over 5 min with continuous stirring. The reaction was allowed to warm to room temperature over 4 h and quenched by addition of Et_3N (0.31 mL, 2.3 mmol), and the solvents were evaporated under reduced pressure. The residue was immediately dissolved in a solution of MeOH/THF/H_2O, 1:2:1 (37 mL), to which was added NaOH (438 mg, 11.0 mmol). The reaction mixture was stirred overnight at room temperature and then acidified to pH ≈ 4 with aqueous HCl 10%. The solvents were evaporated under reduced pressure. The solid residue was purified by C-18 reversed-phase flash chromatography (MeOH/H_2O, 7:3 to 9:1) to afford **21a** (363 mg, 84%, 2 steps) as a white, amorphous powder: $[\alpha]^{25}_D$ +1.2 (c 0.5, MeOH); ^{1}H NMR (Pyr-d_5, 400 MHz) δ 5.05 (1H, d, J = 7.6 Hz, H-1″), 4.98 (1H, d, J = 7.8 Hz, H-1′), 4.83 (1H, d, J = 2.1 Hz, H-29), 4.71 (1H, br s, H-29), 4.67 (1H, m, H-6″), 4.63 (1H, m, H-6′), 4.49 (1H, dd, J = 12.1, 5.1 Hz, H-6″), 4.45 (1H, dd, J = 11.6, 5.3 Hz, H-6′), 4.34 (1H, m, H-3″), 4.34 (1H, m, H-4″), 4.28 (1H, m, H-3′), 4.27 (1H, m, H-4′), 4.14 (1H, m, H-2″), 4.13 (1H, m, H-5″), 4.10 (1H, m, H-28), 4.08 (1H, m, H-2′), 4.03 (1H, m, H-5′), 3.95 (1H, d, J = 9.7 Hz, H-28), 3.43 (1H, dd, J = 11.4, 4.3 Hz, H-3), 1.72 (3H, s, H-30), 1.33 (3H, s, H-23), 1.03 (3H, s, H-27), 1.01 (3H, s, H-24), 0.94 (3H, s, H-26), 0.80 (3H, s, H-25), 0.74 (1H, br d, J = 8.9 Hz, H-5); ^{13}C NMR, see Table 3; HRESIMS m/z 789.4747 [M + Na]$^+$ (calcd for $C_{42}H_{70}O_{12}Na$, 789.4760).

28-*O*-*β*-D-Glucopyranosylbetulinic acid 3*β*-*O*-*β*-D-glucopyranoside (21b). This compound was prepared from the acceptor **2** (50 mg, 0.109 mmol) and the donor **9** (243 mg, 0.328 mmol) in the same manner as that described for compound **21a**. Purification by C-18 reversed-phase flash chromatography (MeOH/H_2O, 7:3 to 17:3) gave **21b** (49 mg, 58%, 2 steps): white, amorphous powder; $[\alpha]^{25}_D$ −6.8 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.49 (1H, d, J = 8.1 Hz, H-1″), 4.71 (1H, br s, H-29), 4.60 (1H, br s, H-29), 4.30 (1H, d, J = 7.6 Hz, H-1′), 3.84 (1H, m, H-6″), 3.83 (1H, m, H-6′), 3.70 (1H, dd, J = 11.9, 3.0 Hz, H-6″), 3.65 (1H, dd, J = 11.9, 5.3 Hz, H-6′), 3.42 (1H, m, H-3″), 3.39 (1H, m, H-4″), 3.38 (1H, m, H-5″), 3.33 (1H, m, H-3′), 3.31 (1H, m, H-2″), 3.28 (1H, m, H-4′), 3.24 (1H, m, H-5′), 3.18 (1H, m, H-2′), 3.15 (1H, m, H-3), 3.01 (1H, td, J = 10.8, 4.5 Hz, H-19), 1.69 (3H, s, H-30), 1.03 (3H, s, H-23), 0.99 (3H, s, H-27), 0.95 (3H, s, H-26), 0.86 (3H, s, H-25), 0.82 (3H, s, H-24); ^{13}C NMR, see Table 3; HRESIMS m/z 803.4537 [M + Na]$^+$ (calcd for $C_{42}H_{68}O_{13}Na$, 803.4552).

28-*O*-α-L-Rhamnopyranosylbetulin 3*β*-*O*-α-L-rhamnopyranoside (22a). This compound was prepared from the acceptor **1** (100 mg, 0.226 mmol) and the donor **6** (421 mg, 0.678 mmol) in the same manner as that described for compound **21a**. Purification by C-18 reversed-phase flash chromatography (MeOH/H_2O, 3:2 to 9:1) gave **22a** (53 mg, 32%, 2 steps): white, amorphous powder; $[\alpha]^{25}_D$ −58.4 (c 0.1, $CHCl_3$/MeOH,

1:1); ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1, 400 MHz) δ 4.76 (1H, br s, H-1′), 4.70 (1H, m, H-1″), 4.70 (1H, m, H-29), 4.59 (1H, m, H-29), 3.88 (1H, m, H-2′), 3.88 (1H, m, H-2″), 3.75 (1H, dd, J = 9.4, 6.2 Hz, H-5″), 3.69 (1H, m, H-3′), 3.69 (1H, m, H-3″), 3.60 (1H, dd, J = 9.4, 6.4 Hz, H-5′), 3.51 (1H, d, J = 9.2 Hz, H-28), 3.43 (1H, m, H-28), 3.40 (1H, m, H-4′), 3.38 (1H, m, H-4″), 3.07 (1H, dd, J = 11.6, 4.8 Hz, H-3), 2.47 (1H, m, H-19), 1.69 (3H, s, H-30), 1.33 (3H, d, J = 6.2 Hz, H-6′), 1.26 (3H, d, J = 6.4 Hz, H-6″), 1.03 (3H, s, H-26), 0.99 (3H, s, H-27), 0.92 (3H, s, H-23), 0.84 (3H, s, H-25), 0.76 (3H, s, H-24), 0.72 (1H, br d, J = 10.0 Hz, H-5); ^{13}C NMR ($CDCl_3/CD_3OD$, 1:1, 100 MHz), see Table 3; HRESIMS *m/z* 757.4843 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{10}Na$, 757.4861).

28-*O*-α-L-Rhamnopyranosylbetulinic acid 3*β*-O-α-L-rhamnopyranoside (22b). This compound was prepared from the acceptor **2** (100 mg, 0.219 mmol) and the donor **6** (408 mg, 0.657 mmol) in the same manner as that described for compound **21a**. Purification by C-18 reversed-phase flash chromatography (MeOH, 7:3 to 17:3) gave **22b** (60 mg, 37%, 2 steps): white, amorphous powder; $[\alpha]^{25}_D$ −47.0 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 6.00 (1H, d, J = 1.6 Hz, H-1″), 4.75 (1H, d, J = 1.3 Hz, H-29), 4.72 (1H, d, J = 1.3 Hz, H-1′), 4.62 (1H, br s, H-29), 3.82 (1H, dd, J = 3.2, 1.6 Hz, H-2′), 3.79 (1H, dd, J = 3.3, 1.9 Hz, H-2″), 3.70 (1H, m, H-5′), 3.67 (1H, m, H-3″), 3.67 (1H, m, H-5″), 3.63 (1H, m, H-3′), 3.46 (1H, t, J = 9.4 Hz, H-4″), 3.36 (1H, t, J = 9.4 Hz, H-4′), 3.07 (1H, dd, J = 11.6, 4.9 Hz, H-3), 3.02 (1H, td, J = 10.7, 4.6 Hz, H-19), 1.71 (3H, s, H-30), 1.27 (3H, d, J = 6.2 Hz, H-6″), 1.22 (3H, d, J = 6.2 Hz, H-6′), 1.02 (3H, s, H-27), 0.94 (3H, s, H-26), 0.93 (3H, s, H-23), 0.87 (3H, s, H-25), 0.77 (3H, s, H-24); ^{13}C NMR, see Table 3; HRESIMS *m/z* 771.4639 $[M + Na]^+$ (calcd for $C_{42}H_{68}O_{11}Na$, 771.4654).

Cell Lines and Culture Conditions. Human lung carcinoma (A549), human colorectal adenocarcinoma (DLD-1), human breast adenocarcinoma (MCF7), human prostate adenocarcinoma (PC-3), and human normal skin fibroblasts (WS1) cell lines were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in minimum essential medium containing Earle's salts and L-glutamine (Mediatech Cellgro, VA), to which were added 10% fetal bovine serum (Hyclone), vitamins (1×), penicillin (100 IU/mL), streptomycin (100 μg/mL), essential amino acids (1×), and sodium pyruvate (1×) (Mediatech Cellgro, VA). Cells were kept at 37 °C in a humidified environment containing 5% CO_2.

Cytotoxicity Assay. Exponentially growing cells were plated in 96-well microplates (Costar, Corning Inc.) at a density of 5×10^3 cells per well in 100 μL of culture medium and were allowed to adhere for 16 h before treatment. Increasing concentrations of each compound in biotech DMSO (Sigma-Aldrich) and the cells were incubated for 48 h. The final concentration of DMSO in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cytotoxicity was assessed using resazurin[55] on an automated 96-well Fluoroskan Ascent F1 plate reader (Labsystems) using excitation and emission wavelengths of 530 and 590 nm, respectively. Fluorescence was proportional to the cellular metabolic activity in each well. Survival percentage was defined as the fluorescence in experimental wells compared to that in control wells after subtraction of blank values. Each experiment was carried out three times in triplicate. IC_{50} results were expressed as means ± standard deviation.

Statistical Analysis. Significant differences of cytotoxicity between samples were determined by Kruskal−Wallis one way analysis of variance on ranks followed by *post hoc* multiple comparisons with the Student−Newman−Keuls method. Probabilities (P) inferior to 0.05 were considered significant. All computations were done using statistical software Sigma-Stat version 3.5.

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References and Notes

(1) Bruneton, J. In *Pharmacognosie, Phytochimie, Plantes Médicinales*; Éditions Technique & Documentation: Paris, 1999.
(2) Sparg, S. G.; Light, M. E.; van Staden, J. *J. Ethnopharmacol.* **2004**, *94*, 219–243.
(3) Baumann, E.; Stoya, G.; Völkner, A.; Richter, W.; Lemke, C.; Linss, W. *Acta Histochem.* **2000**, *102*, 21–35.
(4) Oda, K.; Matsuda, H.; Murakami, T.; Katayama, S.; Ohgitani, T.; Yoshikawa, M. *Biol. Chem.* **2000**, *381*, 67–74.
(5) Yu, B.; Xie, J.; Deng, S.; Hui, Y. *J. Am. Chem. Soc.* **1999**, *121*, 12196–12197.
(6) Suhr, R.; Lahmann, M.; Oscarson, S.; Thiem, J. *Eur. J. Org. Chem.* **2003**, 4003–4011.
(7) Zhang, Y.; Li, Y.; Zhu, S.; Guan, H.; Lin, F.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1753–1759.
(8) Li, M.; Yu, B. *Org. Lett.* **2006**, *8*, 2679–2682.
(9) Peng, W.; Han, X.; Yu, B. *Synthesis* **2004**, *10*, 1641–1647.
(10) Kim, Y.-J.; Wang, P.; Navarro-Villalobos, M.; Rohde, B.; Derryberry, J.; Gin, D. Y. *J. Am. Chem. Soc.* **2006**, *128*, 11906–11915.
(11) Li, C.-X.; Zang, J.; Wang, P.; Zhang, X.-L.; Guan, H.-S.; Li, Y.-X. *Chin. J. Chem.* **2006**, *24*, 509–517.
(12) Cheng, S.; Du, Y.; Bing, F.; Zhang, G. *Carbohydr. Res.* **2008**, *343*, 462–469.
(13) Krasutsky, P. A. *Nat. Prod. Rep.* **2006**, *23*, 919–942.
(14) Sami, A.; Taru, M.; Salme, K.; Jari, Y.-K. *Eur. J. Pharm. Sci.* **2006**, *29*, 1–13.
(15) Gauthier, C.; Legault, J.; Lebrun, M.; Dufour, P.; Pichette, A. *Bioorg. Med. Chem.* **2006**, *14*, 6713–6725.
(16) Thibeault, D.; Gauthier, C.; Legault, J.; Bouchard, J.; Dufour, P.; Pichette, A. *Bioorg. Med. Chem.* **2007**, *15*, 6144–6157.
(17) Klinitová, E.; Křeček, V.; Klinot, J.; Endová, M.; Eisenreichová, J.; Buděšínský, M.; Štícha, M. *Collect. Czech. Chem. Commun.* **1997**, *62*, 1776–1798.
(18) Baglin, I.; Poumaroux, A.; Nour, M.; Tan, K.; Mitaine-Offer, A. C.; Lacaille-Dubois, M. A.; Chauffert, B.; Cavé, C. *J. Enzym. Inhib. Med. Chem.* **2003**, *18*, 111–117.
(19) Ohara, S.; Ohira, T. *J. Wood Sci.* **2003**, *49*, 59–64.
(20) Samoshina, N. F.; Denisenko, M. V.; Denisenko, V. A.; Uvarova, N. I. *Chem. Nat. Compd.* **2003**, *39*, 575–582.
(21) Pakulski, Z. *Pol. J. Chem.* **2005**, *79*, 361–367.
(22) Cmoch, P.; Pakulski, Z.; Swaczynová, J.; Strnad, M. *Carbohydr. Res.* **2008**, *343*, 995–1003.
(23) Sung, T. V.; Peter-Katalinic, J.; Adam, G. *Phytochemistry* **1991**, *30*, 3717–3720.
(24) Cioffi, G.; Braca, A.; Autore, G.; Morelli, I.; Pinto, A.; Venturella, F.; De Tommasi, N. *Planta Med.* **2003**, *69*, 750–756.
(25) Braca, A.; Autore, G.; De Simone, F.; Marzocco, S.; Morelli, I.; Venturella, F.; De Tommasi, N. *Planta Med.* **2004**, *70*, 960–966.
(26) Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Hamanaka, M.; Sakuma, C.; Sashida, Y. *J. Nat. Prod.* **2001**, *64*, 1226–1229.
(27) Ye, W.; Zhang, Q.; Hsiao, W. W. L.; Zhao, S.; Che, C.-T. *Planta Med.* **2002**, *68*, 183–186.
(28) Glebko, L. I.; Krasovskaj, N. P.; Strigina, L. I.; Ulanova, K. P.; Denisenko, V. A.; Dmitrenok, P. S. *Russ. Chem. B.* **2002**, *51*, 1945–1950.
(29) Ziegler, T.; Bien, F.; Jurisch, C. *Tetrahedron: Asymmetry* **1998**, *9*, 765–780.
(30) Fukase, K.; Winarno, H.; Kusumoto, S. *Chem. Express* **1993**, *8*, 409–412.
(31) Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353–3356.
(32) Bliard, C.; Massiot, G.; Nazabadioko, S. *Tetrahedron Lett.* **1994**, *35*, 6107–6108.
(33) Li, T.-S.; Wang, J.-X.; Zheng, X.-J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3957–3965.
(34) Du, Y.; Wei, G.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 2206–2209.
(35) Fletcher, H. G. *Methods Carbohydr. Chem.* **1963**, *2*, 226–228.
(36) Gauthier, C.; Legault, J.; Lavoie, S.; Rondeau, S.; Tremblay, S.; Pichette, A. *Tetrahedron* **2008**, *64*, 7386–7399.
(37) Schmidt, R. R. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
(38) Wang, P.; Zhang, Z.; Yu, B. *J. Org. Chem.* **2005**, *70*, 8884–8889.
(39) Li, C.; Yu, B.; Liu, M.; Hui, Y. *Carbohydr. Res.* **1998**, *306*, 189–195.
(40) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307–3330.
(41) Jermal, A.; Siegel, R.; Ward, E.; Murray, T.; Xu, J.; Smigal, C.; Thun, M. J. *CA Cancer J. Clin.* **2006**, *56*, 106–130.
(42) Kessler, J. H.; Mullauer, F. B.; de Roo, G. M.; Medema, J. P. *Cancer Lett.* **2007**, *251*, 132–145.
(43) Kim, D. S. H. L.; Pezzuto, J. M.; Pisha, E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1707–1712.
(44) Chatterjee, P.; Pezzuto, J. M.; Kouzi, S. A. *J. Nat. Prod.* **1999**, *62*, 761–763.
(45) Urban, M.; Sarek, J.; Tislerova, I.; Dzubak, P.; Hajduch, M. *Bioorg. Med. Chem.* **2005**, *13*, 5527–5535.
(46) Cham, B. E.; Daunter, B. *Cancer Lett.* **1990**, *55*, 221–225.
(47) Chang, L.-C.; Tsai, T.-R.; Wang, J.-J.; Lin, C.-N.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 21–25.
(48) Raz, A.; Lotan, R. *UCLA Symp. Mol. Cell. B* **1988**, *78*, 237–244.

(49) Mody, R.; Shantaram, J.; Chaney, W. *J. Pharmacol. Toxicol.* **1995**, *33*, 1–10.
(50) Jordinson, M.; Calam, J.; Pignatelli, M. *Exp. Opin. Invest. Drugs* **1998**, *7*, 1389–1403.
(51) Yamazaki, N.; Kojima, S.; Bovin, N. V.; André, S.; Gabius, S.; Gabius, H.-J. *Adv. Drug Delivery Rev.* **2000**, *43*, 225–244.
(52) Ambrosi, M.; Cameron, N. R.; Davis, B. G. *Org. Biomol. Chem.* **2005**, *3*, 1593–1608.
(53) Wu, A. M.; Lisowska, E.; Duk, M.; Yang, Z. *Glycoconj. J.* **2008,** 10.1007/s10719-008-9119-7.
(54) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.
(55) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.

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