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Glycosidation of lupane-type triterpenoids as potent in vitro cytotoxic agents

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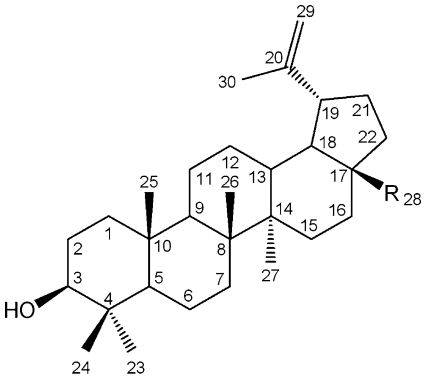
Abstract—The weak hydrosolubility of betulinic acid (**3**) hampers the clinical development of this natural anticancer agent. In order to circumvent this problem and to enhance the pharmacological properties of betulinic acid (**3**) and the lupane-type triterpenes lupeol (**1**), betulin (**2**), and methyl betulinate (**7**), glycosides (β-D-glucosides, α-L-rhamnosides, and α-D-arabinosides) were synthesized and in vitro tested for cytotoxicity against three cancerous (A-549, DLD-1, and B16-F1) and one healthy (WS1) cell lines. The addition of a sugar moiety at the C-3 or C-28 position of betulin (**2**) resulted in a loss of cytotoxicity. In contrast, the 3-O-β-D-glucosidation of lupeol (**1**) improved the activity by 7- to 12-fold (IC_{50} 14–15.0 μM). Moreover, the results showed that cancer cell lines are 8- to 12-fold more sensitive to the 3-*O*-α-L-rhamnopyranoside derivative of betulinic acid (IC_{50} 2.6–3.9 μM, **22**) than the healthy cells (IC_{50} 31 μM). Thus, this study indicates that 3-*O*-glycosides of lupane-type triterpenoids represent an interesting class of potent in vitro cytotoxic agents.

1. Introduction

In the boreal forest of North America, lupeol (**1**), betulin (**2**), and betulinic acid (**3**) (Fig. 1) are found in the external bark of yellow (*Betula alleghaniensis*) and white (*Betula papyrifera*) birches.[1,2] These pentacyclic triterpenes of the lupane-type have recently been investigated by the scientific community for their various pharmacological and medicinal properties.[3–6] Lupeol (**1**) known for its in vivo anti-inflammatory activity[7] exhibited in vitro cytotoxicity against human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431),[8] while it did not affect the growth of tumor cell lines such as human melanoma (MEL-2), human lung carcinoma (A-549), and murine melanoma (B16-F10).[9] Moreover, lupeol (**1**) was found to exhibit a significant antiangiogenic activity on in vitro tube formation of human umbilical vein endothelial cells (HUVEC).[9] In addition, induction of apoptosis (programmed cell death) by lupeol (**1**) was observed in human leukemia HL-60 cells.[10]

Betulinic acid (**3**) is easily synthesized in a two-step process[11,12] by taking advantage of the abundance of betulin (**2**) in the bark of white birches. In addition to various medicinal properties including anti-inflammatory,[13] anti-malarial,[14] and anti-HIV[15] activities, betulinic acid (**3**) possesses a strong in vitro cytotoxicity against a broad panel of human cancers like melanoma,[16] neuroectodermal,[17] and malignant brain[18] tumor cell lines. The in vivo activity was first confirmed by experiments on human melanoma-bearing mice. Indeed, after subcutaneous injection of human MEL-2 cells, betulinic acid



Lupeol **1**: R = CH_3
Betulin **2**: R = CH_2OH
Betulinic acid **3**: R = COOH

Figure 1. Structures of lupane-type triterpenoids.

Keywords: Triterpenes; Betulinic acid; Glycosides; Cytotoxicity.
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(**3**) demonstrated highly effective tumor growth inhibition without inducing any side effects such as weight loss or mortality.[16] Moreover, betulinic acid (**3**) seems to induce apoptosis in cancer cells via the activation of caspases independently of the p53 gene status.[17] Due to the apparent lack of toxicity on normal cells[19] and the favorable therapeutic index, betulinic acid (**3**) is a very promising anticancer agent.[3–6]

However, a major inconvenience for the future clinical development of betulinic acid (**3**) and analogues resides in their poor solubility in aqueous media like blood serum and polar solvents used for bioassays.[3] To circumvent this problem of hydrosolubility and to enhance pharmacological properties, many derivatives were synthesized and evaluated for cytotoxic activity.[20–25] A study showed that C-20 modifications involve the loss of cytotoxicity.[20] Another study demonstrated the importance of the presence of the COOH group since compounds substituted at this position like lupeol (**1**) and methyl betulinate (**7**) were less active on human melanoma than betulinic acid (**3**).[21] Moreover, some C-28 amino acid[22] and C-3 phthalate[23] derivatives of **3** exhibited higher cytotoxic activity against cancer cell lines with improved selective toxicity and water solubility. On the other hand, little work has been carried out on the synthesis and the structure–activity relationships of triterpenoid lupane-type saponins.[26–30] Chatterjee and co-workers[28] obtained the 28-*O*-β-D-glucopyranoside of **3** by microbial transformation with *Cunninghamella* species, while Baglin and co-workers[29] obtained it by organic synthesis. This glucoside did not exhibit any significant in vitro activity on human melanoma (MEL-2)[28] and human colorectal adenocarcinoma (HT-29)[29] cell lines which confirms the importance of the carboxylic acid function to preserve the cytotoxicity.

Our research group is interested in the synthesis of triterpenoid saponins of the lupane-type. Saponins have long been well known for their biological activities including antitumoral and cytotoxic activities.[31,32] Many natural products including triterpenes are present as glycoconjugates. Sugar moieties are known to influence the pharmacokinetic properties of the respective compounds such as absorption, distribution, metabolism, and elimination.[33] Since it is generally accepted that glycosides are more water-soluble than the respective aglycones, glycosidation of triterpenes should increase hydrosolubility and ameliorate pharmacological properties. Furthermore, it is known that cancerous cells need a more significant sugar contribution than normal cells.[34] Consequently, this difference could be exploited to support the absorption of the therapeutic agent by the tumoral site.

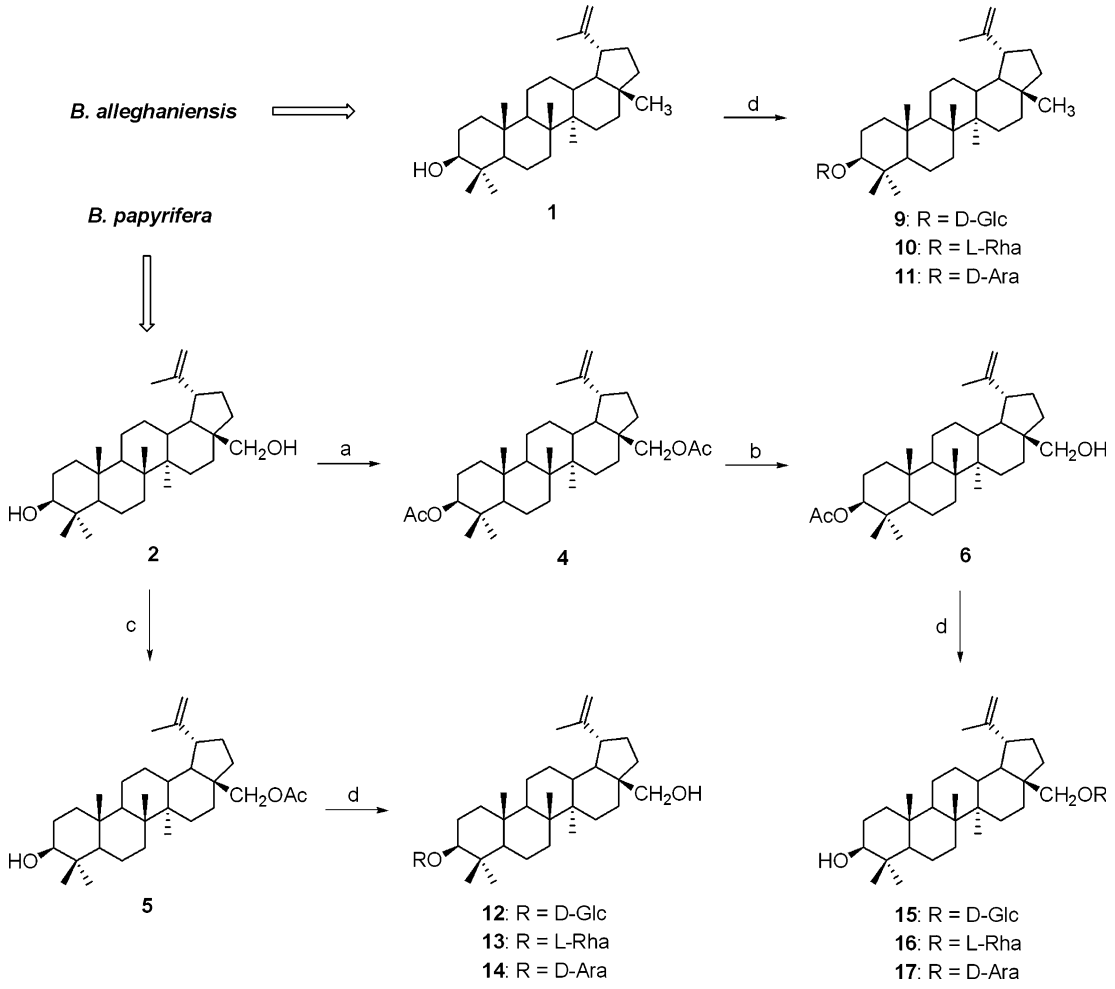
In this study, we report the synthesis of several monodesmosides (β-D-glucoside, α-L-rhamnoside, and α-D-arabinoside) (**9**–**23**) of triterpenoid saponins at the C-3 position for lupeol (**1**), betulinic acid (**3**), and its methyl ester **7**, and at the C-3 and C-28 positions for betulin (**2**). Cytotoxicity of compounds was evaluated on human lung carcinoma (A-549), human colorectal adenocarcinoma (DLD-1), and mouse melanoma (B16-F1). Investigations were also carried out on human normal skin fibroblasts (WS1) to evaluate the differential cytotoxicity with respect to the healthy cells.

2. Results and discussion

2.1. Chemistry

2.1.1. Extraction and synthesis of triterpenes. First of all, the external bark of yellow and white birches was refluxed in $CHCl_3$. Purification of the extracts on silica gel followed by treatment with activated charcoal gave, respectively, the natural triterpenes **1** (1.2%) and **2** (17%). To perform the glycosidation at the C-3 and C-28 positions of **2**, we needed to prepare the corresponding acetates. As the reactivity of the C-28 hydroxyl group of **2** is much higher than the one at C-3, 28-acetoxybetulin (**5**) was obtained in moderate yield (73%) by using an excess of acetic anhydride (Ac_2O) in CH_2Cl_2 during a 24 h period at room temperature. As shown in Scheme 1, diacetylation of **2** with Ac_2O, pyridine, and a catalytic amount of dimethylaminopyridine (DMAP) in CH_2Cl_2 afforded 3,28-diacetoxybetulin (**4**) in excellent yield (95%).[35] Subsequent selective deprotection of the C-28 alcohol using $Mg(OCH_3)_2$ in dry CH_3OH and THF furnished the 3-acetoxybetulin (**6**) in good yield (85%) as previously reported.[36] However, it is important to note that, in the same experimental conditions, contrary to the results of Xu and co-workers, the reaction was complete after 4 h instead of 3 days. As shown in Scheme 2, the methyl ester **7** of the commercially available **3** was synthesized in moderate yield (71%) by treatment with iodomethane in the presence of DBU.[37] Methods used to regenerate the carboxylic acid (NaOH 1 N refluxed in DMF or dioxane and $Ba(OH)_2{\cdot}8H_2O$ in CH_3OH) from methyl betulinate glycosides (**18**–**20**) failed to yield the corresponding betulinic acid glycosides (**21**–**23**). Therefore, another more versatile protection group for the C-28 acid function was considered. To this end, the synthesis of allyl betulinate (**8**) was carried out in good yield (84%) by reaction of **3** using allyl bromide in DMF in the presence of K_2CO_3.[38]

2.1.2. Synthesis of activated sugars. Protection of sugar alcohols (Scheme 3) was achieved by using benzoyl chloride in pyridine with DMAP as catalyst to afford 1,2,3,4,6-penta-*O*-benzoyl-α,β-D-glucopyranose (**24**, 92%), 1,2,3,4-tetra-*O*-benzoyl-α,β-L-rhamnopyranose (**27**, 82%), and 1,2,3,4-tetra-*O*-benzoyl-α,β-D-arabinopyranose (**29**, 89%).[39] Thereafter, bromination (HBr/HOAc 33%) of the benzoylated sugars followed by basic hydrolysis with silver carbonate (Ag_2CO_3) in acetone/H_2O 20:1 allowed the selective deprotection of the anomeric position in good yield for 2,3,4,6-tetra-*O*-benzoyl-α,β-D-glucopyranose (**25**, 86%) and in a quantitative way for L-rhamnose and D-arabinose derivatives.[40] Finally, trichloroacetimidate derivatives **26**[41] (85%), **28**[42] (72%, 2 steps), and **30** (78%, 2 steps) were synthesized from the corresponding 1-OH sugars according to Schmidt's procedure[43] using trichloroacetonitrile (CCl_3CN) and a catalytic amount of cesium carbonate (Cs_2CO_3) in CH_2Cl_2.[44]



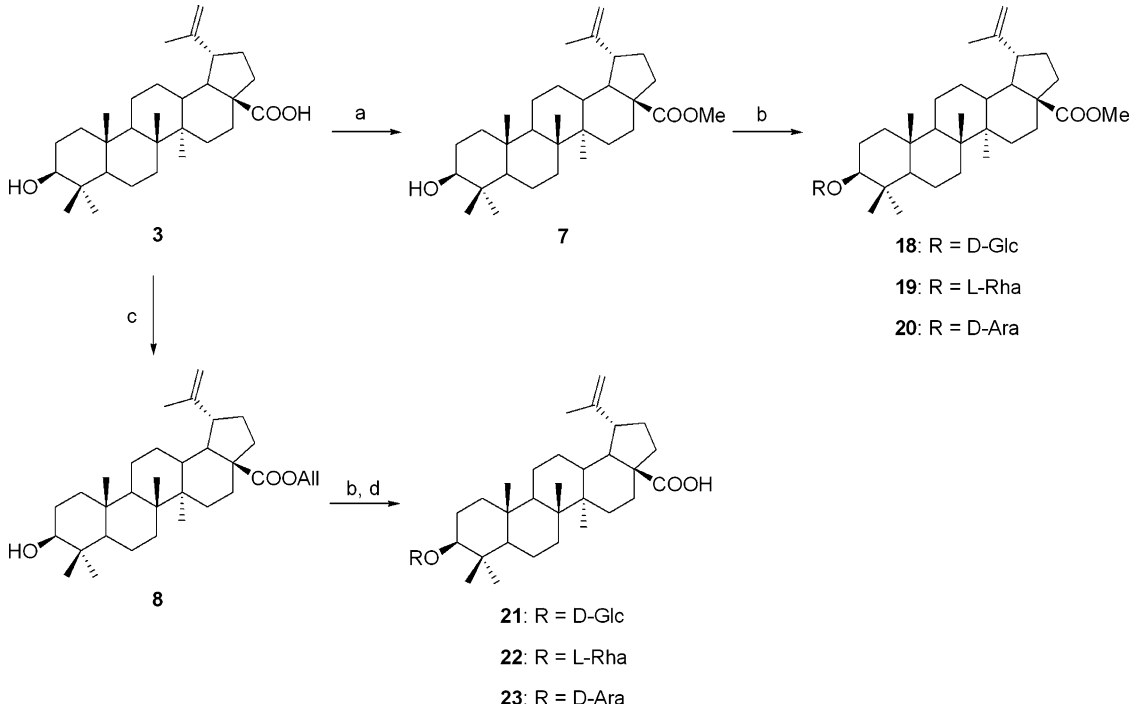
Scheme 1. Reagents and conditions: (a) Ac_2O, Py, DMAP, 0 °C to rt, 5 h; (b) $Mg(OCH_3)_2$, CH_3OH–THF, rt, 4 h; (c) Ac_2O, CH_2Cl_2, rt, 24 h; (d) i—trichloroacetimidate, TMSOTf, 4 Å MS, CH_2Cl_2, rt, 30 min; ii—CH_3OH/THF/H_2O 1:2:1, NaOH 0.25 N, rt, 3–24 h.

2.1.3. Synthesis of glycosides. Glycosidations of the lupane-type triterpenoids were achieved by the reaction of acceptors (**1**, **5**–**8**) with donors (**26**, **28**, and **30**) at room temperature in CH_2Cl_2 under the catalytic promotion of the Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf).[40] Subsequent removal of the protecting groups (benzoyl and acetate) by using NaOH 0.25 N in CH_3OH/THF/H_2O 1:2:1 gave glycosides **9**–**20** (52–87%, 2 steps). Betulinic acid glycosides **21**–**23** (41–50%, 3 steps) were only obtained after the regeneration of the C-28 acid function in the presence of a catalytic amount of tetrakistriphenylphosphine palladium $Pd^0(PPh_3)_4$ and pyrrolidine in dry THF.[38] Since the glycosyl donors contained benzoyl participating neighboring groups, exclusively 1,2-*trans*-glycosides were synthesized as confirmed by ^1H NMR experiments. Indeed, the chemical shifts and the vicinal coupling constants of the anomeric protons were characteristic for β-D-glucosides (δ = 4.22–4.95 ppm, J = 7.7–8.7 Hz), α-L-rhamnosides (δ = 4.72–5.39 ppm, broad singlets), and α-D-arabinosides (δ = 4.06–4.70 ppm, J = 5.6–7.1 Hz).[45] The glycosides showed a greater solubility than corresponding triterpenes in the polar solvents (DMSO and CH_3OH) used for bioassays (data not shown).

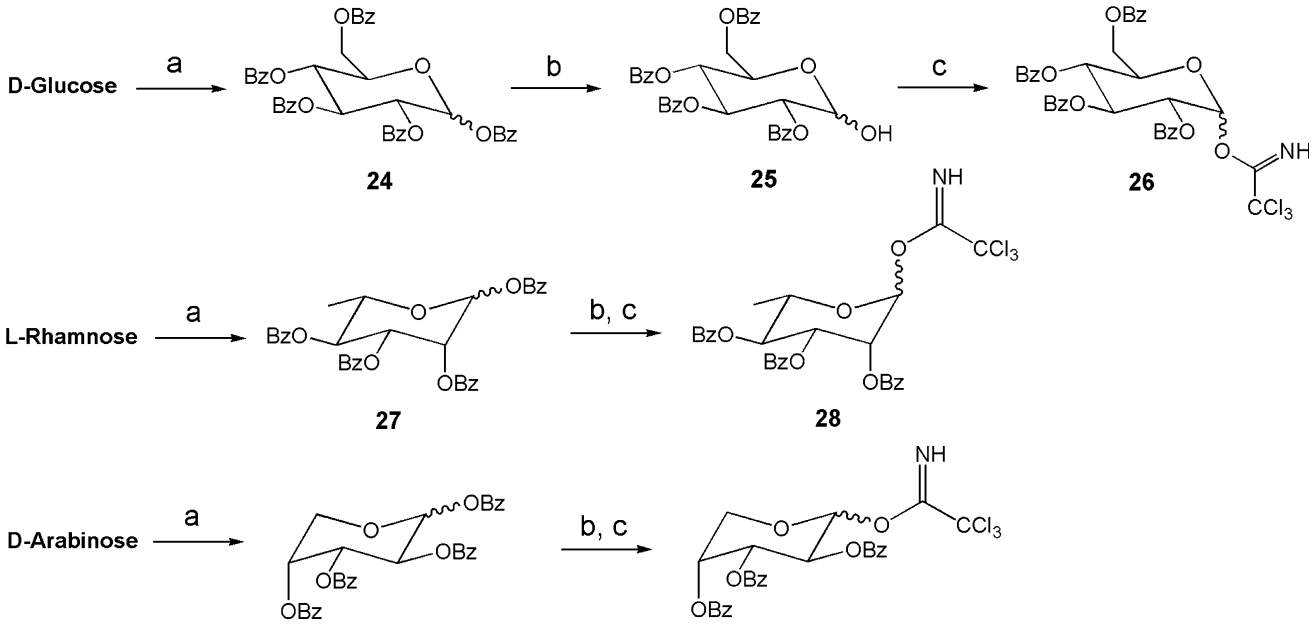
2.2. Cytotoxic activity

The in vitro cytotoxicity of triterpenes (**1**–**8**) and corresponding glycosides (**9**–**23**) was assessed toward human cancer (A-549, DLD-1), mouse melanoma (B16-F1), and human normal skin fibroblast (WS1) cell lines using the resazurin reduction test (RTT test) as previously described in the literature.[46] Measurements of fluorescence were carried out after 48 continuous hours of contact between compounds and cells. Results presented in Table 1 are expressed as the concentration inhibiting 50% of the cell growth (IC_{50}). Known for its activity against A-549, betulinic acid (**3**) was used as a positive control in this experimentation. Based on the IC_{50} values, we considered that compounds with IC_{50} <20 μM were strongly active, those with IC_{50} ranging from ~20 to 75 μM were moderately active and those with IC_{50} ranging from ~75 to 165 μM were weakly active. Otherwise, the compounds were considered as inactive.

2.2.1. Triterpenes. Lupeol (**1**) was found to be weakly cytotoxic against cancer cell lines with IC_{50} ranging from 104 to 165 μM. These results are in good agreement with the group of Moriarity and co-workers.[8] Surprisingly, results demonstrated that, among all test-



Scheme 2. Reagents and conditions: (a) DBU, CH_3I, THF, 0 °C to rt, 24 h; (b) i—trichloroacetimidate, TMSOTf, 4 Å MS, CH_2Cl_2, rt, 30 min; ii—$CH_3OH/THF/H_2O$ 1:2:1, NaOH 0.25 N, rt, 3 h; (c) AllBr, K_2CO_3, 55 °C, 7 h; (d) $Pd^0(PPh_3)_4$, PPh_3, pyrrolidine, THF, 24 h.

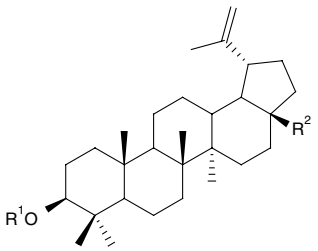


Scheme 3. Reagents and conditions: (a) BzCl, Py, DMAP, 0 °C to rt, 24 h; (b) i—HBr/HOAc 33%, CH_2Cl_2, rt, 2 h; ii—Ag_2CO_3, acetone/H_2O 20:1, rt, 1 h; (c) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 4 h.

ed triterpenes, betulin (**2**) exhibited the strongest cytotoxicity toward cancer lines (IC_{50} 3.80–13.8 μM).[47] In comparison to other well-known compounds like betulinic acid (IC_{50} 10.3 μM, **3**) and methyl betulinate (IC_{50} 19 μM, **7**), betulin (**2**) was 3- to 5-fold more potent against A-549 (IC_{50} 3.80 μM). With regard to acetate derivatives of betulin (**2**), only the 28-acetoxy derivative **5** exerted a moderate cytotoxic activity (IC_{50} 43–75 μM) against cancer lines. Moreover, as we expected, the allyl ester **8** was a totally inactive compound (IC_{50} >225 μM). It is significant to note that tested triterpenes including betulinic acid (**3**) are not selective toward cancer cell lines and inhibit also healthy cells' growth.

2.2.2. Lupeol and betulin glycosides. The 3-O-β-D-glucosidation of **1** to give compound **9** substantially enhanced the cytotoxicity about 7- to 12-fold with IC_{50} values

Table 1. In vitro cytotoxicity of lupane-type triterpenoids and glycosides



Compound	R^1	R^2	Cell line IC_{50} (μM ± SD)[a]			
			A-549[b]	DLD-1[c]	B16-F1[d]	WS1[e]
1	H	CH_3	165 ± 8	125 ± 6	104 ± 6	63 ± 3
2	H	CH_2OH	3.80 ± 0.09	6.6 ± 0.3	13.8 ± 0.5	3.58 ± 0.07
3	H	COOH	10.3 ± 0.4	15.0 ± 0.3	16.1 ± 0.5	12 ± 1
4	Ac	CH_2OAc	>95	>95	>95	>95
5	H	CH_2OAc	75 ± 7	56 ± 4	43 ± 2	44 ± 2
6	Ac	CH_2OH	>253	>253	>253	>253
7	H	$COOCH_3$	19 ± 3	25 ± 4	26 ± 1	19 ± 2
8	H	COOAll	>225	>225	>225	>225
9	Glc	CH_3	14 ± 1	14 ± 1	15.0 ± 0.7	13.3 ± 0.5
10	Rha	CH_3	>178	>178	>178	>178
11	Ara	CH_3	28 ± 2	50 ± 6	27 ± 2	15.8 ± 0.8
12	Glc	CH_2OH	>200	>200	>200	>200
13	Rha	CH_2OH	22 ± 3	50 ± 10	18 ± 1	33 ± 5
14	Ara	CH_2OH	41 ± 3	63 ± 8	38 ± 3	59 ± 5
15	H	CH_2O-Glc	>248	>248	>248	>248
16	H	CH_2O-Rha	>228	>228	>228	>228
17	H	CH_2O-Ara	>175	>175	>175	>175
18	Glc	$COOCH_3$	8.4 ± 3	3.93 ± 0.09	7.1 ± 0.3	9.3 ± 0.2
19	Rha	$COOCH_3$	59 ± 3	>183	55 ± 2	53 ± 2
20	Ara	$COOCH_3$	13.5 ± 0.6	18 ± 1	13.3 ± 0.4	12.5 ± 0.4
21	Glc	COOH	>178	32 ± 9	49 ± 13	> 178
22	Rha	COOH	2.6 ± 0.6	3.9 ± 0.4	3.9 ± 0.4	31 ± 3
23	Ara	COOH	10 ± 2	17 ± 3	11 ± 1	47 ± 5

Glc, β-D-glucopyranose; Rha, α-L-rhamnopyranose; Ara, α-D-arabinopyranose; Ac, acetate; All, allyl.
[a] Data represent mean values (±SD) for three independent experiments.
[b] Human lung carcinoma.
[c] Human colorectal adenocarcinoma.
[d] Mouse melanoma.
[e] Human normal skin fibroblasts.

ranging from 14 to 15.0 μM. In contrast, 3-*O*-α-L-rhamnopyranoside of lupeol (**10**) was inactive (IC_{50} >178 μM), while the D-arabinoside derivative **11** displayed a moderate cytotoxicity (IC_{50} 27–50 μM). As for lupeol (**1**), we anticipated that adding a glucose section at the C-3 position of betulin (**2**) should ameliorate its biological activity. In fact, the cytotoxicity of **2** was lost against all tested cell lines (IC_{50} >200 μM) when it was converted to the 3-*O*-β-D-glucopyranoside **12**. However, the replacement of D-glucose by L-rhamnose or D-arabinose at the same position gave compounds exerting moderate cytotoxicity (IC_{50} 18–50 μM, **13**; 38–63 μM, **14**). In the same way that the 28-*O*-β-D-glucoside of betulinic acid (**3**) did not exhibit any significant activity,[28,29] glycosidation on the C-28 primary alcohol of betulin (**2**) completely suppressed the activity (IC_{50} >248 μM, **15**; >228 μM, **16**; >175 μM, **17**). These results suggest that the C-28 alcohol function of betulin (**2**) is important for the cytotoxicity and, therefore, coupling a sugar moiety is not a desirable modification to carry out at this position.

2.2.3. Betulinic acid and methyl betulinate glycosides. In this study, the most interesting monodesmosides synthesized and evaluated were betulinic acid (**3**) and methyl betulinate (**7**) glycosides (**18**–**23**). Indeed, it appears that 3-*O*-β-D-glucopyranoside of methyl betulinate (**18**) exhibited strong cytotoxicity toward DLD-1 and B16-F1 (IC_{50} 3.93 and 7.1 μM, respectively). The D-arabinoside derivative **20** also displayed an effective activity similar to **3** and **9** (IC_{50} 13.3–18 μM) while the L-rhamnoside derivative **19** exerted moderate activity only against A-549 and B16-F1 (IC_{50} 59 and 55 μM, respectively). Moreover, strong cytotoxicities were obtained for 3-*O*-α-L-rhamnopyranoside (**22**) and 3-*O*-α-D-arabinopyranoside (**23**) of betulinic acid (IC_{50} 2.6–3.9 and 10–17 μM, respectively). In addition to their higher solubility in polar solvents (data not shown), betulinic acid glycosides (**21**–**23**) are the only compounds in this study displaying differential cytotoxicity toward cancer cell lines in comparison to healthy cell lines. Indeed, **22** and **23** exhibit only a moderate activity against WS1 (IC_{50} 31 and 47 μM, respectively), while the 3-*O*-

β-D-glucopyranoside derivative **21** was inactive toward healthy cells (IC_{50} >178 μM). These results show that the glycosidation of betulinic acid (**3**) at the C-3 position gives potent and selective cytotoxic agents contrary to the previously reported inactive 28-*O*-β-D-glucoside derivative.[28,29] It appeared that, for all synthesized glycosides, the acid function at the C-28 position seems to be important for the activity and the selectivity against tested cancer cell lines. Indeed, by adding an α-L-rhamnopyranose moiety at the C-3 position of betulinic acid (**3**), we obtained the strongest in vitro anticancer agent (**22**) of all tested triterpenes and glycosides, while the differential cytotoxicity with respect to the healthy cells increased up to 12-fold.

3. Conclusion

From our in vitro cytotoxic results and in terms of structure–activity relationships, we can conclude that: (i) the glycosides **18** and **22** exert a stronger activity than betulinic acid (**3**) against tested cancerous cell lines; (ii) 3-O- and 28-O-glycosidation of betulin (**2**) decreases the activity; (iii) 3-O-β-D-glucosidation of lupeol (**1**) and methyl betulinate (**7**) enhances the activity; (iv) 3-O-α-L-rhamnosidation decreases the activity except for betulinic acid (**3**); (v) 3-O-α-D-arabinosidation enhances the activity except for betulin (**2**); (vi) 3-O-glycosidation of betulinic acid (**3**) increases the selectivity against tested cancerous cell lines and enhances the activity in the case of L-rhamnose and D-arabinose. Thus, our study indicates that 3-*O*-glycosides of lupane-type triterpenoids represent an interesting class of compounds for further pharmacological studies and preclinical developments. In vivo assessments on tumor-bearing mice are now in progress in our laboratory to evaluate the toxicity and the anticancer activity of the promising 3-*O*-α-L-rhamnopyranoside of betulinic acid (**22**).

4. Experimental

4.1. Chemicals

Air and water sensitive reactions were performed in flame-dried glassware under a nitrogen or argon atmosphere. Moisture sensitive reagents were introduced via a dry syringe. Dichloromethane was distilled from CaH_2. THF was distilled from sodium with benzophenone as indicator of moisture. Betulinic acid (**3**) was purchased from Indofine Chemical Company. Tetrakistriphenylphosphine palladium(0) was prepared as mentioned in the literature[48] and stored under nitrogen. All other chemicals and materials were purchased from Sigma–Aldrich and were used as received. Flash chromatography was carried out using 60–230 mesh silica gel. Analytical thin-layer chromatography was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates and 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, and 20 mL H_2SO_4) with charring. All of the chemical yields are not optimized and generally represent the result of the mean of two experiments. ^1H NMR spectra were recorded at 400 MHz and ^{13}C NMR were recorded at 100 MHz on an Avance 400 Bruker spectrometer equipped with a 5 mm QNP probe. Elucidations of chemical structures were based on ^1H, ^{13}C, DEPT135, COSY, HSQC, and HMBC NMR experiments. Chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quinquet), c (complex), br s (broad singlet), and coupling constants are reported in hertz (Hz). Melting points were determined in capillaries and are uncorrected. Optical rotations were obtained using sodium D line at ambient temperature on a Jasco DIP-360 digital polarimeter. Mass spectral data (HRMS) were obtained at the Department of Chemistry, Queen's University Ontario, Canada.

4.2. Isolation of lupeol (1)

The finely ground external bark (150 g) of the yellow birch (*B. alleghaniensis* Britton), collected in Saguenay, Quebec, Canada, was extracted in $CHCl_3$ (1 L) with a soxhlet apparatus, refluxed for 1 day, and purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH 99:1) to give **1** as a white powder (1.77 g, 1.2%): R_f 0.63 (CH_2Cl_2); mp 213–215 °C, lit.[49] mp 215–216 °C; $[\alpha]_D^{20}$ +19.6 (*c* 1.2, $CHCl_3$), lit.[49] $[\alpha]_D$ +26.4 ($CHCl_3$). ^1H and ^{13}C NMR spectral data of **1** were in agreement with those published in the literature.[6] HR-EI-MS *m*/*z* 426.3854 $[M]^+$ (calcd for $C_{30}H_{50}O$, 426.3862).

4.3. Isolation of betulin (2)

The finely ground external bark (150 g) of the white birch (*B. papyrifera* Marsh.), collected in Saguenay, Quebec, Canada, was soaked in CH_2Cl_2 (1 L), refluxed for 1 day, and purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH 49:1) to give **2** as a white powder (25 g, 17%): R_f 0.17 (CH_2Cl_2); mp 250–252 °C, lit.[49] mp 251–252 °C; $[\alpha]_D^{20}$ +19.1 (*c* 0.67, C_5H_5N), lit.[49] $[\alpha]_D^{15}$ +20.0 (C_5H_5N). ^1H and ^{13}C NMR spectral data of **2** were in agreement with those published in the literature.[50] HR-EI-MS *m*/*z* 442.3804 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 442.3811).

4.4. 3,28-Diacetoxybetulin (4)

Acetic anhydride (4.8 mL, 50 mmol) was added to a cooled solution (ice-water bath) of **2** (7.50 g, 17 mmol) in pyridine (182 mL) with DMAP (100 mg, 0.82 mmol) as catalyst. After stirring at room temperature for 5 h, the mixture was diluted with CH_2Cl_2, then, washed with cold H_2SO_4 3 N, saturated $NaHCO_3$ solution, and brine. The solvents of the dried solution ($MgSO_4$) were evaporated under reduced pressure and the residue was purified by flash chromatography (hexanes to hexanes/EtOAc 97:3) to give **4** as a white crystalline powder (8.48 g, 95%): R_f 0.74 (CH_2Cl_2); mp 216–218 °C, lit.[49] mp 223–224 °C; $[\alpha]_D^{20}$ +19.7 (*c* 1.67, $CHCl_3$), lit.[49] $[\alpha]_D^{20}$ +22. ^1H and ^{13}C NMR spectral data of **4** were in agreement with those published in the literature.[35] HR-ESI-MS *m*/*z* 549.3925 $[M+Na]^+$ (calcd for $C_{34}H_{54}O_4Na$, 549.3920).

4.5. 28-Acetoxybetulin (5)

Acetic anhydride (300 mL, 3.1 mol) was added to a solution of **2** (11.6 g, 26.2 mmol) in CH_2Cl_2 (750 mL). After stirring overnight at room temperature, the mixture was washed exhaustively with saturated $NaHCO_3$ solution and brine. The solvents of the dried solution ($MgSO_4$) were evaporated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH 49:1) to give **5** as a white powder (9.28 g, 73%): R_f 0.31 (CH_2Cl_2); mp 210–212 °C; $[\alpha]_D^{20}$ +8.5 (*c* 1.58, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **5** were in agreement with those published in the literature.[26,35] HR-EI-MS *m/z* 484.3903 $[M]^+$ (calcd for $C_{32}H_{52}O_3$, 484.3916).

4.6. 3-Acetoxybetulin (6)

A solution of $Mg(OCH_3)_2$ in CH_3OH (224 mL, 8%) was added under N_2 to a solution of **4** (6.14 g, 11.7 mmol) in dry THF (181 mL) and dry CH_3OH (542 mL). After stirring for 4 h at room temperature, the mixture was acidified with HCl 10% and extracted with CH_2Cl_2 (3×). Then, the organic layer was washed with saturated $NaHCO_3$ solution and brine. The solvents of the dried solution ($MgSO_4$) were evaporated under reduced pressure and the residue was purified by flash chromatography (hexanes to hexanes/EtOAc 9:1) to give **6** as a white solid (4.80 g, 85%): R_f 0.49 (CH_2Cl_2); mp 258–260 °C, lit.[36] mp 256–258 °C; $[\alpha]_D^{20}$ +25.7 (*c* 0.92, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **6** were in agreement with those published in the literature.[36] HR-EI-MS *m/z* 484.3904 $[M]^+$ (calcd for $C_{32}H_{52}O_3$, 484.3916).

4.7. Methyl betulinate (7)

DBU (0.17 mL, 1.1 mmol) and CH_3I (0.21 mL, 3.3 mmol) were slowly added under N_2 to a cooled solution (ice-water bath) of **3** (502 mg, 1.09 mmol) in dry THF (10 mL). The reaction mixture was stirred overnight at room temperature, then filtered off and washed with dry THF. The filtrate and the combined washings were concentrated to give a yellow solid. This residue was acidified (HCl 6 N) and extracted with CH_2Cl_2 (3×). After that, the organic layer was washed with H_2O, dried ($MgSO_4$) and then the solvents were evaporated under reduced pressure. The resulting residue was purified by flash chromatography (CH_2Cl_2) to give **7** as a white powder (367 mg, 71%): R_f 0.54 (CH_2Cl_2); mp 218–220 °C, lit.[51] 217-220 °C; $[\alpha]_D^{20}$ +1.3 (*c* 0.58, $CHCl_3$), lit.[51] $[\alpha]_D^{25}$ +5 (*c* 0.17, $CHCl_3$), lit.[52] $[\alpha]_D^{26}$ +4.0 (*c* 0.5, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **7** were in agreement with those published in the literature.[52–54] HR-EI-MS *m/z* 470.3744 $[M]^+$ (calcd for $C_{31}H_{50}O_3$, 470.3760).

4.8. Allyl betulinate (8)

Allyl bromide (0.19 mL, 2.2 mmol) and K_2CO_3 (454 mg, 3.28 mmol) were added to a solution of **3** (501 mg, 1.10 mmol) in DMF (7 mL). The reaction mixture was stirred for 7 h at 55 °C. After cooling, EtOAc was added and the organic layer was washed with 1 N HCl. The aqueous layer was extracted with EtOAc (3×) and the combined organic layers were washed with saturated $NaHCO_3$, and brine. After the solution was dried ($MgSO_4$), the solvents were evaporated under reduced pressure. The resulting residue was purified by flash chromatography (CH_2Cl_2) to give **8** as a white crystalline powder (458 mg, 84%): R_f 0.58 (CH_2Cl_2/CH_3OH 99:1); mp 152–154 °C; $[\alpha]_D^{20}$ +3.9 (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 0.77, 0.83, 0.92 (all s, each 3H, H-24, H-25, H-26), 0.97 (s, 6H, H-23, H-27), 1.69 (s, 3H, H-30), 3.02 (m, 1H, H-19), 3.19 (dd, 1H, J = 11.0 Hz, J = 5.1 Hz, H-3), 4.58 (m, 2H, $CH_2CH{=}CH_2$), 4.61 (br s, 1H, H-29α), 4.74 (br s, 1H, H-29β), 5.24 (d, 1H, J = 10.5 Hz, $CH_2CH{=}CH_2$, Hα), 5.35 (d, 1H, J = 17.1 Hz, $CH_2CH{=}CH_2$, Hβ), 5.94 (ddt, 1H, J = 17.1 Hz, J = 10.5 Hz, J = 5.7 Hz, $CH_2CH{=}CH_2$), 0.69–2.28 (all m, remaining protons). ^{13}C NMR ($CDCl_3$) δ: 14.75, 15.44, 16.00, 16.19, 18.33, 19.44, 20.92, 25.56, 27.43, 28.04, 29.68, 30.61, 32.15, 34.36, 37.03, 37.22, 38.24, 38.77, 38.89, 40.77, 42.42, 46.94, 49.48, 50.59, 55.39, 56.59, 64.61 ($CH_2CH{=}CH_2$), 78.91 (C-3), 109.64 (C-29), 118.15 ($CH_2CH{=}CH_2$), 132.56 ($CH_2CH{=}CH_2$), 150.53 (C-20), 175.72 (C-28). HR-ESI-MS *m/z* 497.3985 $[M+H]^+$ (calcd for $C_{33}H_{53}O_3$, 497.3995).

4.9. 1,2,3,4,6-Penta-*O*-benzoyl-α,β-D-glucopyranose (24)

BzCl (77 mL, 666 mmol) was slowly added to a cooled solution (ice-water bath) of D-glucose (20.0 g, 111 mmol) in anhydrous pyridine (280 mL) with DMAP (136 mg, 1.1 mmol) as catalyst. The reaction was performed overnight at room temperature with constant stirring and then quenched with CH_3OH (31 mL). The mixture was diluted with CH_2Cl_2 and washed with cold H_2SO_4 3 N, saturated $NaHCO_3$ solution and brine. The solvents of the dried solution ($MgSO_4$) were evaporated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2) to give **24** as a white solid (71.6 g, 92%): R_f 0.68 (CH_2Cl_2); mp 172–174 °C; $[\alpha]_D^{20}$ +104.9 (*c* 1.25, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **24** were in agreement with those published in the literature.[39,55] HR-ESI-MS *m/z* 723.1818 $[M+Na]^+$ (calcd for $C_{41}H_{32}O_{11}Na$, 723.1842).

4.10. 2,3,4,6-Tetra-*O*-benzoyl-α,β-D-glucopyranose (25)

HBr/HOAc (10 mL, 33%) was added under N_2 to a solution of **24** (10.0 g, 14.3 mmol) in dry CH_2Cl_2 (42 mL). The reaction mixture was stirred at room temperature for 4 h, then, the solution was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered and the solvents were evaporated under reduced pressure. After the residue was dissolved in acetone (75 mL) and water (3 mL), Ag_2CO_3 (6.50 g, 23.6 mmol) was added portionwise. The hydrolysis was performed in 1 h at room temperature with constant stirring, then, the mixture was filtered through a bed of Celite. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH 99:1 to 49:1) to give **25** as a white foam (7.32 g, 86%): R_f 0.28 (CH_2Cl_2/CH_3OH 99:1); mp 116–118 °C, lit.[56] mp 118–120 °C; $[\alpha]_D^{20}$ +70.1 (*c* 1.42, $CHCl_3$), lit.[56] $[\alpha]_D^{22}$ +72.2 (*c* 0.5,

$CHCl_3$). ^1H and ^{13}C NMR spectral data of **25** were in agreement with those published in the literature.[41,56] HR-ESI-MS *m/z* 619.1567 $[M+Na]^+$ (calcd for $C_{34}H_{28}O_{10}Na$, 619.1580).

4.11. 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranose trichloroacetimidate (26)

CCl_3CN (6 mL, 59.8 mmol) was added to a solution of **25** (5.81 g, 9.74 mmol) and Cs_2CO_3 (315 mg, 0.97 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred for 4 h at room temperature and then filtered off. The solvents of the filtrate were evaporated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2) to give **26** as a white crystalline powder (6.13 g, 85%): R_f 0.64 (CH_2Cl_2/CH_3OH 99:1); $[\alpha]_D^{20}$ +76.5 (*c* 1.67, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **26** were in agreement with those published in the literature.[41] HR-ESI-MS *m/z* 778.0410 $[M+K]^+$ (calcd for $C_{36}H_{28}NO_{10}Cl_3K$, 778.0415).

4.12. 1,2,3,4-Tetra-*O*-benzoyl-α,β-L-rhamnopyranose (27)

This compound was prepared from L-rhamnose (2.05 g, 12.5 mmol) in the same manner as that described for compound **24**. Purification by flash chromatography (CH_2Cl_2) afforded **27** as a white crystalline powder (5.95 g, 82%): R_f 0.65 (CH_2Cl_2); $[\alpha]_D^{20}$ +33.6 (*c* 0.25, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 1.52 (d, 3H, J = 6.2 Hz, H-6), 4.20 (m, 1H, H-5), 5.85 (t, 1H, J = 9.6 Hz, H-4), 5.91 (dd, 1H, J = 10.0 Hz, J = 3.2 Hz, H-3), 6.24 (d, 1H, J = 3.0 Hz, H-2), 6.54 (br s, 1H, H-1), 7.20–7.25 (m, 2H, H-Ar), 7.28–7.41 (m, 5H, H-Ar), 7.44–7.54 (m, 4H, H-Ar), 7.58–7.64 (m, 1H, H-Ar), 7.88–7.92 (m, 2H, H-Ar), 7.97–8.05 (m, 4H, H-Ar), 8.23–8.27 (m, 2H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 17.84 (C-6), 69.88 (C-5), 71.44 (C-2), 71.62 (C-3), 71.75 (C-4), 91.38 (C-1), 128.39–133.75 (C-Ar), 164.27, 165.51, 165.74, 165.85 (4× *C*O). HR-ESI-MS *m/z* 603.1613 $[M+Na]^+$ (calcd for $C_{34}H_{28}O_9Na$, 603.1631).

4.13. 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranose trichloroacetimidate (28)

HBr/HOAc (2.3 mL, 33%) was added at room temperature under N_2 to a solution of **27** (2.31 g, 3.98 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 2 h, then, the solution was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered and the solvents were evaporated under reduced pressure. After the residue was dissolved in acetone (19 mL) and water (0.8 mL), Ag_2CO_3 (1.50 g, 5.44 mmol) was added portionwise. The hydrolysis was performed in 1 h at room temperature with constant stirring, then, the mixture was filtered through a bed of Celite. The filtrate was concentrated under reduced pressure and dissolved in CH_2Cl_2 (50 mL). Cs_2CO_3 (130 mg, 0.40 mmol) was added, followed by CCl_3CN (2.4 mL, 23.9 mmol), and the reaction mixture was stirred for 4 h at room temperature. The mixture was then filtered off, concentrated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2) to give **28** as a white crystalline powder (1.78 g, 72%, 2 steps): R_f 0.74 (CH_2Cl_2); $[\alpha]_D^{20}$ +83.6 (*c* 1.33, $CHCl_3$), lit.[42] $[\alpha]_D^{20}$ +97.5 (*c* 1.0, $CHCl_3$). ^1H and ^{13}C NMR spectral data of **28** were in agreement with those published in the literature.[42] HR-ESI-MS *m/z* 658.0189 $[M+K]^+$ (calcd for $C_{29}H_{24}NO_8Cl_3K$, 658.0204).

4.14. 1,2,3,4-Tetra-*O*-benzoyl-α,β-D-arabinopyranose (29)

This compound was prepared from D-arabinose (4.92 g, 32.8 mmol) in the same manner as that described for compound **24**. Purification by flash chromatography (CH_2Cl_2) afforded **29** as a white crystalline powder (16.5 g, 89%): R_f 0.59 (CH_2Cl_2); $[\alpha]_D^{20}$ −274.2 (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 4.21 (dd, 1H, J = 13.4 Hz, J = 1.8 Hz, H-5α), 4.44 (d, 1H, J = 13.0 Hz, H-5β), 5.93 (s, 1H, H-4), 6.10 (br s, 2H, H-2, H-3), 6.90 (br s, 1H, H-1), 7.26–7.34 (m, 4H, H-Ar), 7.42–7.56 (m, 6H, H-Ar), 7.61–7.68 (m, 2H, H-Ar), 7.88–7.93 (m, 4H, H-Ar), 8.13–8.18 (m, 4H, H-Ar). ^{13}C NMR ($CDCl_3$) δ: 63.07 (C-5), 67.82 (C-2), 68.23 (C-3), 69.53 (C-4), 91.12 (C-1), 128.44–133.89 (C-Ar), 164.73, 165.62, 165.76, 165.79 (4× *C*O). HR-ESI-MS *m/z* 589.1457 $[M+Na]^+$ (calcd for $C_{33}H_{26}O_9Na$, 589.1474).

4.15. 2,3,4-Tri-*O*-benzoyl-α-D-arabinopyranose trichloroacetimidate (30)

This compound was prepared from **29** (5.70 g, 10.1 mmol) in the same manner as that described for compound **28**. Purification by flash chromatography (CH_2Cl_2) afforded **30** as a white foam (4.76 g, 78%, 2 steps): R_f 0.55 (CH_2Cl_2); $[\alpha]_D^{20}$ −182.8 (*c* 1.00, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 4.19 (dd, 1H, J = 13.3 Hz, J = 2.0 Hz, H-5α), 4.43 (d, 1H, J = 12.8 Hz, H-5β), 5.88 (m, 1H, H-4), 6.02 (ddd, 2H, J = 16.7 Hz, J = 10.7 Hz, J = 3.0 Hz, H-2, H-3), 6.83 (d, 1H, J = 3.0 Hz, H-1), 7.26–7.33 (m, 2H, H-Ar), 7.34–7.40 (m, 2H, H-Ar), 7.44–7.55 (m, 4H, H-Ar), 7.60–7.66 (m, 1H, H-Ar), 7.84–7.88 (m, 2H, H-Ar), 7.96–8.00 (m, 2H, H-Ar), 8.09–8.15 (m, 2H, H-Ar), 8.64 (br s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 63.18 (C-5), 68.00 (d, C-2, C-3), 69.45 (C-4), 90.89 (*C*Cl_3), 94.35 (C-1), 128.38–133.57 (C-Ar), 160.80 (*C*=NH), 165.59, 165.66, 165.69 (3× *C*O). HR-ESI-MS *m/z* 644.0076 $[M+K]^+$ (calcd for $C_{28}H_{22}NO_8Cl_3K$, 644.0048).

4.16. 3-*O*-β-D-Glucopyranoside of lupeol (9)

The acceptor **1** (1.01 g, 2.34 mmol) and the donor **26** (2.60 g, 3.52 mmol) were stirred in dry CH_2Cl_2 (80 mL) for 1 h with 4 Å MS. At this time, TMSOTf (24 μL, 0.13 mmol) was added under Ar while keeping rigorous anhydrous conditions. The reaction was usually performed in 30 min, then quenched by addition of Et_3N (0.3 mL). The solvents were evaporated under reduced pressure and the resulting residue was immediately dissolved in a NaOH 0.25 N solution of CH_3OH/THF/H_2O 1:2:1 (240 mL). The reaction mixture was stirred at room temperature for 2 h, dissolved in CH_2Cl_2, and washed with HCl 10% and brine. Once the solution was dried ($MgSO_4$), the solvents were

evaporated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) to give **9** as a white powder (1.38 g, 90%, 2 steps): R_f 0.24 (CH_2Cl_2/CH_3OH 9:1); mp 176–178 °C; $[\alpha]_D^{20}$ +7.9 (*c* 0.50, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 0.79, 0.80, 0.83, 0.93, 0.99, 1.02 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-28), 1.68 (s, 3H, H-30), 2.37 (m, 1H, H-19), 2.63 (br s, 4H, 4× O*H*), 3.13 (dd, 1H, *J* = 11.2 Hz, *J* = 4.8 Hz, H-3), 3.36 (m, 1H, H′-5), 3.42 (t, 1H, *J* = 8.3 Hz, H′-2), 3.58 (q, 2H, *J* = 8.7 Hz, H′-3, H′-4), 3.80 (dd, 1H, *J* = 11.8 Hz, *J* = 4.2 Hz, H′-6α), 3.86 (dd, 1H, *J* = 12.0 Hz, *J* = 3.1 Hz, H′-6β), 4.36 (d, 1H, *J* = 7.7 Hz, H′-1), 4.57 (br s, 1H, H-29α), 4.69 (br s, 1H, H-29β), 0.67–1.92 (all m, remaining protons). ^{13}C NMR ($CDCl_3$) δ: 14.70, 16.15, 16.38, 16.74, 18.16, 18.35, 19.50, 21.00, 25.26, 26.48, 27.60, 28.09, 30.02, 34.46, 35.74, 37.02, 38.20, 38.93, 39.35, 40.15, 40.99, 42.95, 43.17, 48.15, 48.45, 50.57, 55.77, 61.94 (C′-6), 69.69 (C′-4), 73.98 (C′-2), 75.29 (C′-5), 76.51 (C′-3), 90.29 (C-3), 105.32 (C′-1), 109.54 (C-29), 151.08 (C-20). HR-ESI-MS *m*/*z* 611.4267 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_6Na$, 611.4287).

4.17. 3-*O*-α-L-Rhamnopyranoside of lupeol (10)

This compound was prepared from the acceptor **1** (502 mg, 1.18 mmol) and the donor **28** (1.09 g, 1.76 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **10** as a white powder (485 mg, 72%, 2 steps): R_f 0.33 (CH_2Cl_2/CH_3OH 9:1); mp 214–216 °C; $[\alpha]_D^{20}$ −17.9 (*c* 0.50, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 0.75, 0.79, 0.83, 0.90, 0.94, 1.02 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-28), 1.28 (d, 3H, *J* = 6.1 Hz, H′-6), 1.69 (s, 3H, H-30), 2.38 (m, 1H, H-19), 3.07 (dd, 1H, *J* = 11.3 Hz, *J* = 4.8 Hz, H-3), 3.43 (t, 1H, *J* = 9.2 Hz, H′-4), 3.77 (t, 1H, *J* = 5.2 Hz, H′-3), 3.81 (dd, 1H, *J* = 9.0 Hz, *J* = 6.1 Hz, H′-5), 3.95 (br s, 1H, H′-2), 4.57 (br s, 1H, H-29α), 4.69 (br s, 1H, H-29β), 4.82 (br s, 1H, H′-1), 0.68–1.93 (all m, remaining protons). ^{13}C NMR ($CDCl_3$) δ: 14.55, 15.98, 16.15, 16.25, 17.35 (C′-6), 18.01, 18.30, 19.33, 20.95, 25.14, 25.52, 27.44, 28.19, 29.86, 34.25, 35.59, 36.89, 38.05, 38.64, 39.06, 40.01, 40.85, 42.83, 43.02, 48.00, 48.31, 50.40, 55.45, 67.65 (C′-5), 71.26 (C′-2), 71.98 (C′-3), 74.00 (C′-4), 89.71 (C-3), 101.67 (C′-1), 109.33 (C-29), 151.01 (C-20). HR-ESI-MS *m*/*z* 595.4335 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_5Na$, 595.4338).

4.18. 3-*O*-α-D-Arabinopyranoside of lupeol (11)

This compound was prepared from the acceptor **1** (251 mg, 0.59 mmol) and the donor **30** (531 mg, 0.88 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **11** as a white solid (286 mg, 87%, 2 steps): R_f 0.33 (CH_2Cl_2/CH_3OH 9:1); mp 212–214 °C; $[\alpha]_D^{20}$ +26.8 (*c* 1.25, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 0.77, 0.79, 0.84, 0.92, 1.00, 1.02, 1.68 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-28, H-30), 2.38 (m, 1H, H-19), 2.64 (br s, 3H, 3× O*H*), 3.26 (dd, 1H, *J* = 11.9 Hz, *J* = 3.8 Hz, H-3), 3.54 (d, 1H, *J* = 11.4 Hz, H′-5α), 3.65 (m, 1H, H′-3), 3.68 (m, 1H, H′-2), 3.93 (br s, 1H, H′-4), 3.94 (d, 1H, *J* = 11.4 Hz, H′-5β), 4.34 (d, 1H, *J* = 5.9 Hz, H′-1), 4.57 (br s, 1H, H-29α), 4.68 (br s, 1H, H-29β), 0.70–1.92 (all m, remaining protons). ^{13}C NMR ($CDCl_3$) δ: 14.47, 15.98, 16.10, 16.39, 18.00, 18.30, 19.32, 20.96, 23.01, 25.13, 27.41, 28.20, 29.84, 34.26, 35.56, 37.03, 38.02, 38.22, 38.39, 40.00, 40.88, 42.82, 43.02, 47.98, 48.30, 50.39, 55.84, 64.83 (C′-5), 67.49 (C′-4), 71.62 (C′-3), 72.68 (C′-2), 84.59 (C-3), 99.53 (C′-1), 109.33 (C-29), 151.01 (C-20). HR-ESI-MS *m*/*z* 581.4163 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_5Na$, 581.4181).

4.19. 3-*O*-β-D-Glucopyranoside of betulin (12)

This compound was prepared from the acceptor **5** (500 mg, 1.03 mmol) and the donor **26** (1.15 g, 1.55 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **12** as a white crystalline powder (406 mg, 65%, 2 steps): R_f 0.21 (CH_2Cl_2/CH_3OH 9:1); mp 192–194 °C; $[\alpha]_D^{20}$ +2.7 (*c* 0.58, CH_3OH). ^1H NMR (CD_3OD) δ: 0.84, 0.88, 1.02, 1.05, 1.08, 1.69 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 2.42 (m, 1H, H-19), 3.16 (dd, 1H, *J* = 11.2 Hz, *J* = 5.0 Hz, H-3), 3.18 (t, 1H, *J* = 9.8 Hz, H′-2), 3.25 (m, 1H, H′-5), 3.28 (t, 1H, *J* = 11.7 Hz, H′-4), 3.28 (d, 1H, *J* = 11.7 Hz, H-28α), 3.28 (dd, 1H, *J* = 11.9 Hz, *J* = 5.1 Hz, H′-6α), 3.33 (t, 1H, *J* = 9.8 Hz, H′-3), 3.74 (d, 1H, *J* = 11.7 Hz, H-28β), 3.84 (dd, 1H, *J* = 11.9 Hz, *J* = 1.9 Hz, H′-6β), 4.31 (d, 1H, *J* = 7.8 Hz, H′-1), 4.58 (br s, 1H, H-29α), 4.69 (br s, 1H, H-29β), 0.74–1.98 (all m, remaining protons). ^{13}C NMR (CD_3OD) δ: 15.22, 16.54, 16.77, 16.82, 19.28, 19.38, 21.99, 26.62, 27.19, 28.17, 28.41, 30.37, 30.84, 35.10, 35.47, 38.02, 38.70, 40.00, 40.28, 42.16, 43.81, 48.53, 49.25, 50.03, 51.83, 57.10, 60.35 (C-28), 62.79 (C′-6), 71.64 (C′-4), 75.66 (C′-2), 77.68 (C′-5), 78.27 (C′-3), 90.79 (C-3), 106.74 (C′-1), 110.26 (C-29), 151.87 (C-20). HR-ESI-MS *m*/*z* 627.4218 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4236).

4.20. 3-*O*-α-L-Rhamnopyranoside of betulin (13)

This compound was prepared from the acceptor **5** (252 mg, 0.52 mmol) and the donor **28** (484 mg, 0.78 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **13** as a white crystalline powder (159 mg, 52%, 2 steps): R_f 0.29 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ −20.3 (*c* 0.50, CH_3OH). ^1H NMR (CD_3OD) δ: 0.79, 0.88, 0.94, 1.02, 1.08 (all s, each 3H, H-23, H-24, H-25, H-26, H-27), 1.22 (d, 3H, *J* = 6.3 Hz, H′-6), 1.69 (s, 3H, H-30), 2.42 (m, 1H, H-19), 3.07 (dd, 1H, *J* = 11.3 Hz, *J* = 4.6 Hz, H-3), 3.28 (d, 1H, *J* = 10.9 Hz, H-28α), 3.36 (t, 1H, *J* = 9.5 Hz, H′-4), 3.63 (dd, 1H, *J* = 9.5 Hz, *J* = 3.2 Hz, H′-3), 3.70 (m, 1H, H′-5), 3.74 (d, 1H, *J* = 10.9 Hz, H-28β), 3.82 (br s, 1H, H′-2), 4.57 (br s, 1H, H-29α), 4.68 (br s, 1H, H-29β), 4.72 (br s, 1H, H′-1), 0.76–1.95 (all m, remaining protons). ^{13}C NMR (CD_3OD) δ: 15.20, 16.51, 16.72, 16.77, 17.83 (C′-6), 19.34, 19.38, 21.98, 26.58, 26.76, 28.14, 28.61, 30.34, 30.82, 35.09, 35.40, 38.06, 38.68, 39.82, 40.15, 42.15,

43.82, 48.53, 49.24, 50.00, 51.77, 56.79, 60.33 (C-28), 69.88 (C′-5), 72.48 (C′-2), 72.50 (C′-3), 74.07 (C′-4), 90.36 (C-3), 104.43 (C′-1), 110.25 (C-29), 151.86 (C-20). HR-ESI-MS *m/z* 611.4266 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_6Na$, 611.4287).

4.21. 3-*O*-α-D-Arabinopyranoside of betulin (14)

This compound was prepared from the acceptor **5** (250 mg, 0.52 mmol) and the donor **30** (442 mg, 0.78 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **14** as a white powder (196 mg, 66%, 2 steps): R_f 0.29 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ +17.4 (*c* 0.25, CH_3OH). ^1H NMR (C_5D_5N) *δ*: 0.75, 0.84, 0.95, 1.05, 1.22, 1.75 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 2.61 (m, 1H, H-19), 3.42 (dd, 1H, *J* = 11.4 Hz, *J* = 4.2 Hz, H-3), 3.64 (d, 1H, *J* = 10.1 Hz, H-28α), 3.80 (d, 1H, *J* = 11.0 Hz, H′-5), 4.07 (d, 1H, *J* = 10.1 Hz, H-28β), 4.18 (dd, 1H, *J* = 8.7 Hz, *J* = 2.8 Hz, H′-3), 4.32 (br s, 1H, H′-4), 4.34 (d, 1H, *J* = 11.0 Hz, H′-5), 4.39 (t, 1H, *J* = 7.9 Hz, H′-2), 4.70 (d, 1H, *J* = 7.1 Hz, H′-1), 4.74 (br s, 1H, H-29α), 4.88 (br s, 1H, H-29β), 4.99 (br s, 3H, 3× O*H*), 0.72–2.42 (all m, remaining protons). ^{13}C NMR (C_5D_5N) *δ*: 14.90, 16.12, 16.25, 16.91, 18.65, 19.26, 21.06, 23.86, 25.70, 27.54, 28.55, 29.98, 29.99, 30.02, 34.58, 34.87, 37.56, 38.80, 41.08, 41.21, 42.98, 48.35, 48.53, 49.13, 50.61, 56.20, 59.41 (C-28), 67.05 (C′-5), 69.61 (C′-4), 72.55 (C′-2), 74.79 (C′-3), 84.93 (C-3), 102.98 (C′-1), 109.93 (C-29), 151.25 (C-20). HR-ESI-MS *m/z* 587.4143 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_6Na$, 597.4131).

4.22. 28-*O*-β-D-Glucopyranoside of betulin (15)

This compound was prepared from the acceptor **6** (501 mg, 1.03 mmol) and the donor **26** (1.15 g, 1.55 mmol) in the same manner as that described for compound **9** except for the basic hydrolysis reaction time (overnight). Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **15** as a white powder (338 mg, 54%, 2 steps): R_f 0.21 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ −12.8 (*c* 0.25, CH_3OH). ^1H NMR (CD_3OD) *δ*: 0.76, 0.87, 0.96, 1.01, 1.09, 1.69 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 2.46 (m, 1H, H-19), 3.13 (dd, 1H, *J* = 11.1 Hz, *J* = 4.9 Hz, H-3), 3.19 (t, 1H, *J* = 8.4 Hz, H′-2), 3.28 (d, 1H, *J* = 4.7 Hz, H′-5), 3.28 (d, 1H, *J* = 6.0 Hz, H′-4), 3.36 (t, 1H, *J* = 8.9 Hz, H′-3), 3.61 (d, 1H, *J* = 9.5 Hz, H-28α), 3.68 (dd, 1H, *J* = 11.8 Hz, *J* = 5.0 Hz, H′-6α), 3.73 (d, 1H, *J* = 9.5 Hz, H-28β), 3.89 (d, 1H, *J* = 11.6 Hz, H′-6β), 4.22 (d, 1H, *J* = 7.7 Hz, H′-1), 4.57 (br s, 1H, H-29α), 4.68 (br s, 1H, H-29β), 0.71–2.14 (all m, remaining protons). ^{13}C NMR (CD_3OD) *δ*: 15.33, 16.18, 16.67, 16.75, 19.46, 19.50, 22.03, 26.66, 28.08, 28.40, 28.66, 30.69, 30.89, 35.51, 35.87, 38.32, 38.97, 40.00, 40.09, 42.18, 43.86, 46.96, 49.31, 50.17, 51.89, 56.85, 62.87 (C′-6), 68.91 (C-28), 71.77 (C′-4), 75.29 (C′-2), 77.96 (C′-5), 78.21 (C′-3), 79.70 (C-3), 105.35 (C′-1), 110.23 (C-29), 152.00 (C-20). HR-ESI-MS *m/z* 627.4229 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4236).

4.23. 28-*O*-α-L-Rhamnopyranoside of betulin (16)

This compound was prepared from the acceptor **6** (250 mg, 0.52 mmol) and the donor **28** (480 mg, 0.77 mol) in the same manner as that described for compound **9** except for the basic hydrolysis reaction time (overnight). Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **16** as a white powder (203 mg, 67%, 2 steps): R_f 0.31 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ −42.9 (*c* 0.83, CH_3OH). ^1H NMR (C_5D_5N) *δ*: 0.87, 0.95, 0.98, 1.03, 1.22, 1.73 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 1.73 (d, 3H, *J* = 6.3 Hz, H′-6), 2.60 (m, 1H, H-19), 3.45 (m, 1H, H-3), 3.61 (d, 1H, *J* = 9.4 Hz, H-28α), 3.83 (d, 1H, *J* = 9.4 Hz, H-28β), 4.22 (c, 1H, H′-5), 4.33 (t, 1H, *J* = 9.2 Hz, H′-4), 4.51 (dd, 1H, *J* = 9.1 Hz, *J* = 2.9 Hz, H′-3), 4.63 (br s, 1H, H′-2), 4.73 (br s, 1H, H-29α), 4.88 (br s, 1H, H-29β), 5.39 (br s, 1H, H′-1), 0.79-2.12 (all m, remaining protons). ^{13}C NMR (C_5D_5N) *δ*: 14.89, 16.12, 16.37, 16.43, 18.74 (C′-6), 19.32, 21.00, 25.64, 27.55, 27.55, 28.31, 28.66, 30.33, 30.48, 34.59, 35.39, 37.46, 37.68, 39.27, 39.53, 41.15, 42.93, 47.31, 48.07, 49.07, 50.71, 55.83, 66.18 (C-28), 70.06 (C′-5), 72.45 (C′-2), 73.14 (C′-3), 73.94 (C′-4), 78.08 (C-3), 102.30 (C′-1), 110.11 (C-29), 150.89 (C-20). HR-ESI-MS *m/z* 611.4268 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_6Na$, 611.4287).

4.24. 28-*O*-α-D-Arabinopyranoside of betulin (17)

This compound was prepared from the acceptor **6** (250 mg, 0.52 mmol) and the donor **30** (469 mg, 0.77 mmol) in the same manner as that described for compound **9** except for the basic hydrolysis reaction time (overnight). Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **17** as a white crystalline powder (178 mg, 60%, 2 steps): R_f 0.43 (CH_2Cl_2/CH_3OH 9:1); mp 204–206 °C; $[\alpha]_D^{20}$ +4.6 (*c* 0.25, CH_3OH). ^1H NMR (DMSO-d_6) *δ*: 0.65, 0.76, 0.87, 0.93, 0.97, 1.63 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 2.40 (m, 1H, H-19), 2.96 (m, 1H, H-3), 2.99 (d, 1H, *J* = 9.3 Hz, H-28α), 3.32 (m, 1H, H′-3), 3.33 (m, 1H, H′-2), 3.35 (d, 1H, *J* = 11.8 Hz, H′-5α), 3.61 (m, 1H, H′-4), 3.66 (dd, 1H, *J* = 11.8 Hz, *J* = 3.4 Hz, H′-5b), 3.89 (d, 1H, *J* = 9.3 Hz, H-28β), 4.06 (d, 1H, *J* = 5.6 Hz, H′-1), 4.54 (br s, 1H, H-29α), 4.67 (br s, 1H, H-29β), 0.62-1.94 (all m, remaining protons). ^{13}C NMR (DMSO-d_6) *δ*: 14.58, 15.67, 15.82, 15.90, 17.97, 18.76, 20.35, 24.74, 26.67, 27.18, 28.11, 29.29, 29.46, 33.76, 34.03, 36.68, 37.00, 38.25, 38.51, 40.45, 42.19, 46.60, 47.33, 48.33, 49.83, 54.86, 64.80 (C′-5), 66.33 (C-28), 67.40 (C′-4), 70.59 (C′-2), 72.60 (C′-3), 76.80 (C-3), 103.81 (C′-1), 109.77 (C-29), 150.17 (C-20). HR-ESI-MS *m/z* 597.4156 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_6Na$, 597.4131).

4.25. 3-*O*-β-D-Glucopyranoside of methyl betulinate (18)

This compound was prepared from the acceptor **7** (251 mg, 0.53 mmol) and the donor **26** (593 mg, 0.80 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **18** as a white crystalline powder (189 mg, 56%, 2 steps): R_f 0.24

(CH_2Cl_2/CH_3OH 9:1); mp 196–198 °C, lit.[27] mp 197–200 °C; $[\alpha]_D^{20}$ −6.6 (*c* 0.50, $CHCl_3$), lit.[27] $[\alpha]_D$ −3 (*c* 0.38, CH_3OH). ^1H NMR (C_5D_5N) δ: 0.75, 0.94, 0.98, 1.02, 1.30, 1.72 (s, 3H, H-23, H-24, H-25, H-26, H-27, H-30), 3.30 (m, 1H, H-19), 3.40 (dd, 1H, J = 11.7 Hz, J = 4.3 Hz, H-3), 3.70 (s, 3H, $COOCH_3$), 4.01 (m, 1H, H′-5), 4.05 (t, 1H, J = 8.3 Hz, H′-2), 4.23 (t, 1H, J = 8.8 Hz, H′-4), 4.26 (t, 1H, J = 8.5 Hz, H′-3), 4.41 (dd, 1H, J = 11.6 Hz, J = 5.4 Hz, H′-6α), 4.59 (dd, 1H, J = 11.6 Hz, J = 2.2 Hz, H′-6β), 4.72 (br s, 1H, H-29α), 4.88 (br s, 1H, H-29β), 4.95 (d, 1H, J = 7.7 Hz, H′-1), 0.73–2.45 (all m, remaining protons). ^{13}C NMR (C_5D_5N) δ: 14.80, 16.16, 16.32, 16.84, 18.42, 19.37, 21.05, 25.90, 26.76, 28.13, 30.04, 30.91, 32.31, 34.64, 37.08, 37.08, 38.49, 38.99, 39.63, 40.98, 42.67, 47.54, 49.75, 50.69, 51.33 ($COOCH_3$), 55.87, 56.77, 63.04 (C′-6), 71.84 (C′-4), 75.82 (C′-2), 78.35 (C′-5), 78.79 (C′-3), 88.81 (C-3), 106.92 (C′-1), 110.12 (C-29), 150.82 (C-20), 176.45 (C-28). HR-ESI-MS *m/z* 655.4164 $[M+Na]^+$ (calcd for $C_{37}H_{60}O_8Na$, 655.4186).

4.26. 3-*O*-α-L-Rhamnopyranoside of methyl betulinate (19)

This compound was prepared from the acceptor **7** (201 mg, 0.43 mmol) and the donor **28** (398 mg, 0.64 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **19** as a white powder (176 mg, 67%, 2 steps): R_f 0.24 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ −17.1 (*c* 0.42, $CHCl_3$). ^1H NMR (C_5D_5N) δ: 0.77 (s, 6H, H-25, H-26), 0.89, 0.96, 1.00 (all s, each 3H, H-23, H-24, H-27), 1.65 (d, 3H, J = 5.4 Hz, H′-6), 1.72 (s, 3H, H-30), 3.14 (dd, 1H, J = 11.7 Hz, J = 4.3 Hz, H-3), 3.30 (m, 1 H, H-19), 3.70 (s, 3H, $COOCH_3$), 4.29 (m, 1H, H′-4), 4.32 (m, 1H, H′-5), 4.49 (m, 1H, H′-3), 4.72 (br s, 1H, H′-2), 4.72 (br s, 1H, H-29α), 4.88 (br s, 1H, H-29β), 5.32 (br s, 1H, H′-1), 0.66–2.45 (all m, remaining protons). ^{13}C NMR (C_5D_5N) δ: 14.77, 16.14, 16.27, 16.54, 18.52 (C′-6), 19.35, 21.05, 21.13, 25.88, 26.05, 28.13, 30.02, 30.90, 32.29, 33.71, 34.56, 37.07, 38.46, 38.80, 39.28, 40.96, 42.65, 47.53, 49.73, 50.66, 51.34 ($COOCH_3$), 55.61, 56.77, 69.87 (C′-5), 72.51 (C′-2), 72.91 (C′-3), 74.12 (C′-4), 88.51 (C-3), 104.42 (C′-1), 110.13 (C-29), 150.80 (C-20), 176.44 (C-28). HR-ESI-MS *m/z* 639.4223 $[M+Na]^+$ (calcd for $C_{37}H_{60}O_7Na$, 639.4237).

4.27. 3-*O*-α-D-Arabinopyranoside of methyl betulinate (20)

This compound was prepared from the acceptor **7** (200 mg, 0.42 mmol) and the donor **30** (387 mg, 0.64 mmol) in the same manner as that described for compound **9**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 47:3) afforded **20** as a white powder (169 mg, 66%, 2 steps): R_f 0.24 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ +22.7 (*c* 0.42, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 0.75, 0.81, 0.90, 0.93, 0.98, 1.68 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 3.00 (m, 1H, H-19), 3.02 (br s, 3H, 3× O*H*), 3.23 (dd, 1H, J = 11.8 Hz, J = 3.8 Hz, H-3), 3.52 (d, 1H, J = 11.4 Hz, H′-5α), 3.66 (s, 3H, $COOCH_3$), 3.66 (m, 1H, H′-3), 3.70 (m, 1H, H′-2), 3.93 (m, 1H, H′-4), 3.95 (d, 1H, J = 9.4 Hz, H′-5β), 4.31 (d, 1H, J = 6.1 Hz, H′-1), 4.59 (br s, 1H, H-29α), 4.73 (br s, 1H, H-29β), 0.68-2.22 (all m, remaining protons). ^{13}C NMR ($CDCl_3$) δ: 14.76, 16.09, 16.23, 16.54, 18.42, 19.51, 21.04, 23.15, 25.63, 28.32, 29.78, 30.73, 32.29, 34.44, 37.11, 37.18, 38.34, 38.37, 38.54, 40.85, 42.51, 47.10, 49.59, 50.63, 51.44 ($COOCH_3$), 56.02, 56.69, 65.10 (C′-5), 67.80 (C′-4), 71.69 (C′-3), 72.85 (C′-2), 84.81 (C-3), 99.79 (C′-1), 109.72 (C-29), 150.74 (C-20), 176.81 (C-28). HR-ESI-MS *m/z* 625.4073 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_7Na$, 625.4080).

4.28. 3-*O*-β-D-Glucopyranoside of betulinic acid (21)

The acceptor **8** (107 mg, 0.22 mmol) and the donor **26** (239 mg, 0.32 mmol) were stirred in dry CH_2Cl_2 (10 mL) for 1 h with 4 Å MS. At this time, TMSOTf (3 μL, 0.01 mmol) was added under Ar while keeping rigorous anhydrous conditions. The reaction was usually performed in 30 min, then quenched by addition of Et_3N (50 μL). The solvents were evaporated under reduced pressure and the resulting residue was immediately dissolved in a NaOH 0.25 N solution of CH_3OH/THF/H_2O 1:2:1 (30 mL). The reaction mixture was stirred at room temperature for 2 h, dissolved in CH_2Cl_2, and washed with HCl 10% and brine. Once the solution was dried ($MgSO_4$), the solvents were evaporated under reduced pressure to give an oily residue. It was dissolved in a solution of PPh_3 (32 mg, 0.121 mmol) and pyrrolidine (34 μL, 0.403 mmol) in dry THF (1 mL), then $Pd^0(PPh_3)_4$ (70 mg, 0.060 mmol) was added and the reaction mixture was stirred overnight at room temperature. After evaporation of the solvent under reduced pressure, the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 4:1) to give **21** as a white powder (63 mg, 47%, 3 steps): R_f 0.38 (CH_2Cl_2/CH_3OH 4:1); mp 234–236 °C; $[\alpha]_D^{20}$ +1.3 (*c* 0.33, CH_3OH). ^1H NMR (C_5D_5N) δ: 0.73, 0.97, 1.01, 1.09, 1.30, 1.77 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 3.41 (dd, 1H, J = 11.6 Hz, J = 4.0 Hz, H-3), 3.54 (m, 1H, H-19), 4.02 (m, 1H, H′-5), 4.05 (t, 1H, J = 11.1 Hz, H′-2), 4.24 (m, 1H, H′-4), 4.26 (m, 1H, H′-3), 4.42 (dd, 1H, J = 11.6 Hz, J = 5.2 Hz, H′-6α), 4.60 (d, 1H, J = 11.1 Hz, H′-6β), 4.75 (br s, 1H, H-29α), 4.93 (br s, 1H, H-29β), 4.95 (d, 1H, J = 7.8 Hz, H′-1), 0.73–2.69 (all m, remaining protons). ^{13}C NMR (C_5D_5N) δ: 14.84, 16.31, 16.35, 16.82, 18.44, 19.43, 21.15, 26.05, 26.76, 28.19, 30.25, 31.18, 32.85, 34.72, 37.11, 37.57, 38.56, 39.00, 39.63, 41.07, 42.83, 47.76, 49.71, 50.77, 55.88, 56.62, 63.03 (C′-6), 71.84 (C′-4), 75.82 (C′-2), 78.34 (C′-5), 78.78 (C′-3), 88.82 (C-3), 106.92 (C′-1), 109.95 (C-29), 151.29 (C-20), 178.87 (C-28). HR-ESI-MS *m/z* 641.4019 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.29. 3-*O*-α-L-Rhamnopyranoside of betulinic acid (22)

This compound was prepared from the acceptor **8** (100 mg, 0.20 mmol) and the donor **28** (187 mg, 0.30 mmol) in the same manner as that described for compound **21**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 4:1) afforded **22** as a white solid (50 mg, 41%, 3 steps): R_f 0.18 (CH_2Cl_2/CH_3OH 9:1);

mp >200 °C; $[\alpha]_D^{20}$ −22.8 (*c* 0.42, CH_3OH). ^1H NMR (C_5D_5N) δ: 0.75, 0.76, 0.89, 1.02, 1.07 (all s, each 3H, H-23, H-24, H-25, H-26, H-27), 1.66 (d, 3H, J = 5.0 Hz, H′-6), 1.77 (s, 3H, H-30), 3.16 (dd, 1H, J = 11.5 Hz, J = 4.0 Hz, H-3), 3.53 (m, 1H, H-19), 4.29 (m, 1H, H′-4), 4.31 (m, 1H, H′-5), 4.48 (m, 1H, H′-3), 4.58 (br s, 1H, H′-2), 4.75 (br s, 1H, H-29α), 4.93 (br s, 1H, H-29β), 5.33 (br s, 1H, H′-1), 0.67–2.71 (all m, remaining protons). ^{13}C NMR (C_5D_5N) δ: 14.83, 16.28, 16.36, 16.54, 18.49, 18.53 (C′-6), 19.44, 21.18, 25.80, 26.06, 28.15, 30.26, 31.20, 32.86, 34.68, 37.13, 37.58, 38.56, 38.84, 39.30, 41.07, 42.84, 47.77, 49.73, 50.77, 55.65, 56.64, 69.88 (C′-5), 72.52 (C′-2), 72.93 (C′-3), 74.15 (C′-4), 88.53 (C-3), 104.42 (C′-1), 109.97 (C-29), 151.29 (C-20), 178.88 (C-28). HR-ESI-MS *m*/*z* 625.4057 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_7Na$, 625.4080).

4.30. 3-*O*-α-D-Arabinopyranoside of betulinic acid (23)

This compound was prepared from the acceptor **8** (102 mg, 0.21 mmol) and the donor **30** (187 mg, 0.31 mmol) in the same manner as that described for compound **21**. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 49:1 to 4:1) afforded **23** as a white powder (60 mg, 50%, 3 steps): R_f 0.19 (CH_2Cl_2/CH_3OH 9:1); mp >200 °C; $[\alpha]_D^{20}$ +14.0 (*c* 1.00, CH_3OH). ^1H NMR (C_5D_5N) δ: 0.71, 0.81, 1.01, 1.07, 1.21, 1.78 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30), 3.42 (dd, 1H, J = 11.6 Hz, J = 4.0 Hz, H-3), 3.53 (m, 1H, H-19), 3.80 (d, 1H, J = 11.0 Hz, H′-5α), 4.18 (dd, 1H, J = 8.7 Hz, J = 2.7 Hz, H′-3), 4.33 (br s, 1H, H′-4), 4.34 (d, 1H, J = 11.0 Hz, H′-5β), 4.39 (t, 1H, J = 7.9 Hz, H′-2), 4.67 (d, 1H, J = 7.0 Hz, H′-1), 4.77 (br s, 1H, H-29α), 4.94 (br s, 1H, H-29β), 0.73-2.72 (all m, remaining protons). ^{13}C NMR (C_5D_5N) δ: 14.80, 16.20, 16.33, 16.86, 18.62, 19.40, 21.16, 23.84, 26.04, 28.53, 30.22, 31.15, 32.83, 34.71, 37.29, 37.56, 38.53, 38.78, 38.81, 41.08, 42.81, 47.75, 49.72, 50.76, 56.25, 56.60, 67.02 (C′-5), 69.58 (C′-4), 72.51 (C′-2), 74.75 (C′-3), 84.93 (C-3), 102.97 (C′-1), 109.96 (C-29), 151.30 (C-20), 178.82 (C-28). HR-ESI-MS *m*/*z* 611.3908 $[M+Na]^+$ (calcd for $C_{35}H_{56}O_7Na$, 611.3924).

4.31. Cell lines and culture conditions

Human lung carcinoma (A-549), human colon adenocarcinoma (DLD-1), human normal fibroblasts (WS1), and mice melanoma (B16-F1) 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% foetal bovine serum (Hyclone), vitamins (1×), penicillin (100 IU/mL) and 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.

4.32. 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 DMSO (Sigma–Aldrich) were then added (100 μL per well) 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[46] 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 triplicata. IC_{50} results were expressed as means ± standard deviation.

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