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Synthesis and structure–activity relationship study of cytotoxic germanicane- and lupane-type 3β-*O*-monodesmosidic saponins starting from betulin☆

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Abstract—Germanicane-type triterpenes allobetulin (**3**) and 28-oxoallobetulin (**4**) can be obtained by the Wagner-Meerwein rearrangement of the more available lupane-type triterpenes betulin (**1**) and betulinic acid (**2**), respectively. The medical uses of betulinic acid (**2**) and its derivatives are limited because of their poor hydrosolubility and pharmacokinetics properties. In order to overcome this major problem, we synthesized and studied the in vitro anticancer activity of a series of 3β-*O*-monodesmosidic saponins derived from betulin (**14**–**16**), betulinic acid (**20**–**22**), allobetulin (**23**–**28**) and 28-oxoallobetulin (**29**–**34**) based on six different natural sugar residues (D-glucose, L-rhamnose, D-arabinose, D-galactose, D-mannose and D-xylose). This structure–activity relationship study confirmed that betulinic acid saponins are generally better in vitro anticancer agents than those derived from betulin with the exception of betulin 3β-*O*-α-D-mannopyranoside (**15**) which exerted a potent cytotoxic activity against lung carcinoma (A-549) and colorectal adenocarcinoma (DLD-1) human cell lines with IC_{50} ranging from 7.3 to 10.1 μmol/L. Furthermore, although the synthesis of novel germanicane-type saponins was carried out with success, the bioactivity measured for these glycosides was not as high as we anticipated since only the 3β-*O*-β-D-glucopyranoside and 3β-*O*-β-D-galactopyranoside of allobetulin (**23**, **24**) showed moderate anticancer activity (IC_{50} 30–40 μmol/L).

1. Introduction

Lupane-type derivatives have attracted increased attention in the past decade[1] since they exhibit a broad range of biological and medicinal properties such as anticancer,[2] antitumoral,[3,4] anti-inflammatory,[5] anti-HIV,[6] anti-malarial[7] and antifeedant[8] activities. Among these lupanes, betulin (**1**) and betulinic acid (**2**) (Fig. 1) are natural pentacyclic triterpenes that can be found in the external bark of various *Betula* species such as *B. papyrifera*.[9] These compounds and more especially betulin (**1**) are regarded by the scientific community as accessible, abundant and valuable bioactive natural products which are present in the inner and the outer birch bark residues.[10] Because of its selective anti-melanoma activity and its favourable therapeutic index,[3] betulinic acid (**2**) is currently undergoing clinical trials at the National Cancer Institute (NCI). Nevertheless, the medical uses of betulinic acid (**2**) and its derivatives in the pharmaceutical industry are limited because of their poor hydrosolubility and pharmacokinetic properties (absorption, distribution, metabolism and elimination).[11] To overcome this major problem, some laboratories have undertaken the synthesis of more water-soluble betulinic acid (**2**) derivatives. For example, it was reported that the introduction of polar moieties at the C-3 and C-28 positions such as amino acids[12] and phthalates[13] enhances, in certain cases, the hydrosolubility and the anticancer activity of betulin (**1**) and betulinic acid (**2**).

Allobetulin (**3**) and 28-oxoallobetulin (**4**) (Fig. 1) are members of the germanicane-type triterpene family,[14] a rare class of natural compounds. In contrast to the well

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☆ This article is dedicated to the memory of Linda Grenon, secretary at the UQAC Fundamental Sciences Department, who passed away from lung cancer while the preparation of this article was in progress.
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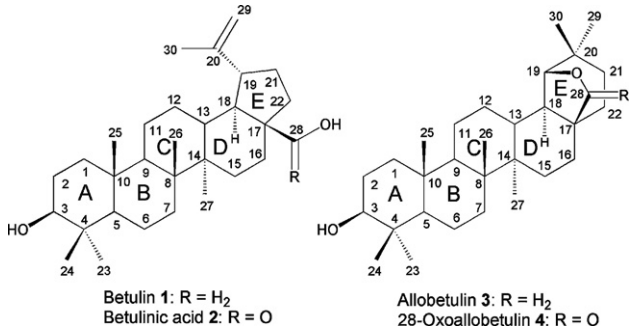
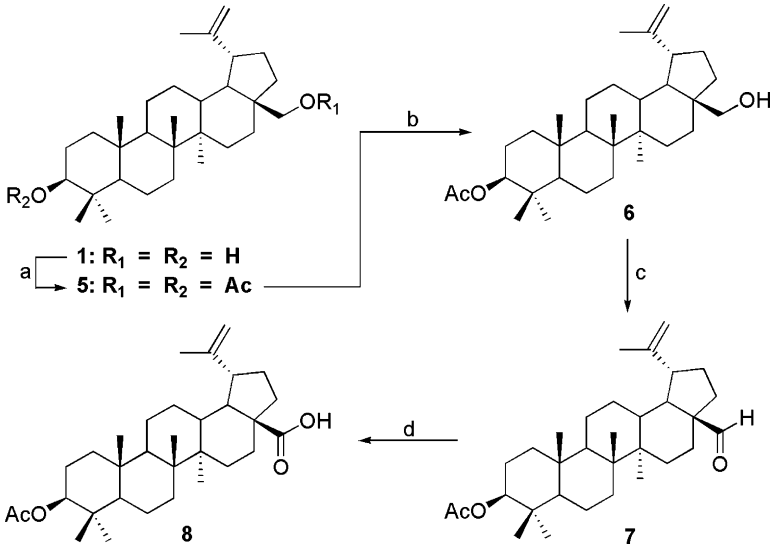


Figure 1. Structures of lupane (**1**,**2**) and germanicane-type (**3**,**4**) triterpenes.

distributed oleanane-type triterpenes in the plant kingdom,[15] the stereochemistry of the hydrogen atom at C-18 is in the α-configuration for the germanicanes rather than the β-configuration. Triterpenes **3** and **4** can be obtained by the Wagner-Meerwein rearrangement of the more abundant betulin (**1**) and betulinic acid (**2**), respectively, in the presence of Lewis or solid acids.[16,17] Although the syntheses of allobetulin (**3**) and oxyallobetulin (**4**) and analogues were already reported in the literature,[17–24] few studies were reported regarding the development of their derivatives for potential therapeutic applications.[25,26] This is probably due to the hydrophobic character of these compounds which complicates the solubilization and the formulation in several biological assays.

Triterpenoid glycosides, saponins, are widely distributed throughout the plant kingdom.[27] They are considered responsible for the bioactivity, including cytotoxic and anticancer activities, of several medicinal plants.[28,29] The synthesis of glycosides is an interesting approach to enhance the hydrosolubility and, consequently, the pharmacological and pharmacokinetic properties of lead compounds.[30] Nonetheless, few studies exist in the literature regarding the synthesis and the biological evaluation of germanicane-[25,31–33] and lupane-type[34–38] saponins. To the best of our knowledge and with respect to the germanicane-type triterpenes, only 3β-*O*-2-deoxy-α-glycopyranosides[25,31] and 3β-*O*-β-D-glucopyranoside[32,33] of allobetulin (**3**) were synthesized and no screening of their bioactivities was reported. Moreover, natural saponins based on betulin (**1**), betulinic acid (**2**), allobetulin (**3**) and 28-oxoallobetulin (**4**) aglycones are especially rare.[39,40] Braca et al.[40] reported the isolation of bidesmosidic saponins from the aerial parts of *Schefflera rotundifolia* where the most active constituents have, interestingly, betulinic acid (**2**) as a residue.

Recently, we focused our attention on the synthesis of 3β-*O*-monodesmosidic lupane-type saponins.[41] Our results showed that adding a sugar moiety at the C-3 position can give stronger in vitro anticancer agents than betulinic acid (**2**). Moreover, glycosides of betulinic acid (**2**) showed a selectivity on the cancerous cells up to 12-fold higher than on healthy cells. These promising results prompted us to extend our study to the germanicane-type allobetulin (**3**) and 28-oxoallobetulin (**4**). In order to establish meaningful structure–activity relationship (SAR) and to modulate the pharmacokinetic properties of the triterpenes (**1**–**4**), we wanted to synthesize glycosides incorporating other natural sugar moieties. Hence, we report here the synthesis of several 3β-*O*-monodesmosidic saponins derived from lupane-(**14**–**16**, **20**–**22**) and germanicane-type (**23**–**34**) triterpenes based on six different natural sugar residues (D-glucose, L-rhamnose, D-arabinose, D-galactose, D-mannose and D-xylose). Cytotoxicity of triterpenes and glycosides was assessed against lung carcinoma (A-549), colorectal adenocarcinoma (DLD-1) and normal skin fibroblasts (WS1) human cell lines. Also, in silico calculations of pharmacokinetic properties were carried out using the ADME prediction program QikProp[42,43] version 2.5 in order to evaluate important pharmaceutical parameters such as lipophilicity (log *P*) and aqueous solubility (log *S*).



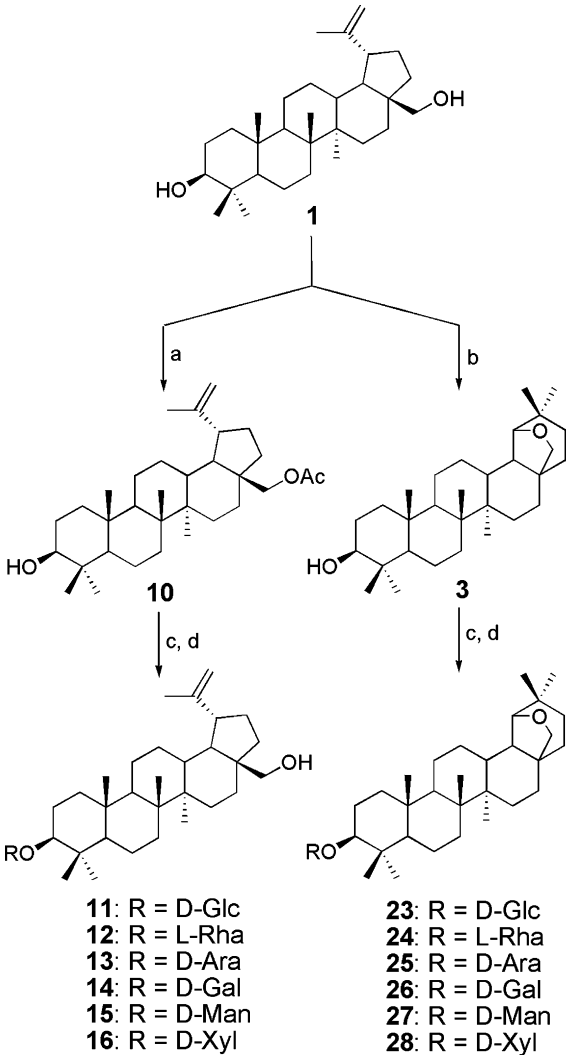
Scheme 1. Synthesis of 3-acetylbetulinic acid (**8**). Reagents and conditions: (a) Ac_2O (2.5 equiv), DMAP (0.1 equiv), pyridine, rt, quantitative; (b) Al(*i*-OPr)$_3$ (5 equiv), *i*-PrOH, reflux 1.5 h, 86%; (c) PCC (2 equiv), CH_2Cl_2, rt, 2 h, 96%; (d) $NaClO_2/NaH_2PO_4$, *t*-BuOH/THF/2-methyl-2-butene, 0 °C to rt, 60 min, 81%.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 3-acetylbetulinic acid (8). The main source of betulin (**1**) used in this study was the available *Betula papyrifera* March. widely distributed in the boreal forest of North America. Finely powdered outer bark was heated under reflux in chloroform or methylene chloride to provide crude betulin (**1**). This extract was purified following the Eckerman and Ekman[44] procedure. Recrystallized betulin (**1**) was the starting material used directly for the synthesis of allobetulin (**3**) and 3-acetylbetulinic acid (**8**) while the latter was the precursor of allyl betulinate (**9**) and 28-oxoallobetulin (**4**). The synthetic pathway employed for the synthesis of 3-acetylbetulinic acid (**8**) (Scheme 1) was inspired by the procedure developed by Krasutsky et al. with slight modifications.[45] Initially, betulin (**1**) was acetylated at the C-3 and C-28 positions with acetic anhydride (Ac_2O) and 4-dimethylaminopyridine (DMAP) in anhydrous pyridine to afford betulin 3,28-diacetate (**5**) in quantitative yield without further purification. Thereafter, compound **5** was deacetylated at the C-28 position in 86% isolated yield in the presence of five equivalents of aluminium isopropoxide. Third, the alcohol **6** was successively converted into aldehyde **7** using pyridinium chlorochromate (PCC) as oxidizing agent in 96% yield.[46] Finally, the crude aldehyde **7** was directly oxidized to 3-acetylbetulinic acid (**8**) by the combined action of sodium chlorite ($NaClO_2$) and sodium dihydrogenophosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) in a *t*-BuOH/THF/2-methyl-2-butene mixture. This procedure was developed by Clive et al.[47] and adapted for substrate **7** to afford compound **8** in only 60–70 min at room temperature in 81% isolated yield. To the best of our knowledge, this synthetic pathway (Scheme 1) is the most efficient methodology for an easy access to 3-acetylbetulinic acid (**8**) and its derivatives.[48]

2.1.2. Synthesis of saponins. The first series of glycosides (**14**–**16**, **23**–**28**) were synthesized according to the reaction sequence shown in Scheme 2. In order to avoid multiple glycosylation reactions, betulin (**1**) was first acetylated at the C-28 position with Ac_2O/DMAP in pyridine to give betulin 28-acetate (**10**). Allobetulin (**3**) was obtained from the Wagner-Meerwein rearrangement of **1** by the action of $Fe(NO_3)_3/SiO_2$ (1:4) in refluxed CH_2Cl_2. Aglycone acceptors **10** and **3** were coupled with the appropriate trichloroacetimidate sugar donors[49] (Fig. 2) under the catalytic promotion of the Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf).[50] Thereafter, deprotection of the acetyl and benzoyl groups (NaOH, MeOH/THF/H_2O 1:2:1) affording betulin (**14**–**16**) and allobetulin (**23**–**28**) saponins in isolated yields ranging from 19% to 49% and 67% to 89%, respectively (two steps). The second series of glycosides (**20**–**22**, **29**–**34**) came from 3-acetylbetulinic acid (**8**) and were synthesized following to the reaction sequence shown in Scheme 3. In order to avoid the formation of by-products, the C-28 allyl ester **9** was first prepared as already reported by our group.[41] 28-Oxoallobetulin (**4**) was obtained from the Wagner-Meerwein rearrangement by the action of $FeCl_3/SiO_2$ (1:4) on the substrate **8** in refluxed CH_2Cl_2. Thereafter, the synthesis of glycosides with aglycone acceptors **9** and **4** was achieved using the same glycosylation protocol mentioned above. Deprotection of the benzoyl groups afforded 28-oxoallobetulin saponins (**29**–**34**) in isolated yields ranging from 20% to 70% (two steps). Betulinic acid saponins (**20**–**22**) were obtained after the supplementary deprotection of the allylic function under the catalytic action of tetrakistriphenylphosphine palladium(0) in isolated yields ranging from 23% to 58% (three steps). The synthesis of other betulin (**11**–**13**) and betulinic acid (**17**–**19**) glycosides was previously reported by our group.[41] As we expected, the presence of benzoyl protecting groups in position 2 of the sugar trichloroacetimidates directed the anomeric selectivity of the glycosidation reaction[51] to give exclusively 1,2-*trans*-monodesmosides (β-D-glucopyranosides, α-L-rhamnopyranosides, α-D-arabinopyranosides, β-D-galactopyr-



Scheme 2. Synthesis of betulin (**11**–**16**) and allobetulin (**23**–**28**) saponins. Reagents and conditions: (a) Ac_2O (1.05 equiv), pyridine, DMAP (0.1 equiv), rt, 2 h, 73%; (b) $Fe(NO_3)_3/SiO_2$, DCM, reflux, 45 min, 72%; (c) trichloroacetimidate (1.5 equiv), TMSOTf (0.05 equiv), 4 Å MS, DCM, rt, 1.5 h; (d) NaOH 0.25 M, MeOH/THF/H_2O, rt, 2 h.

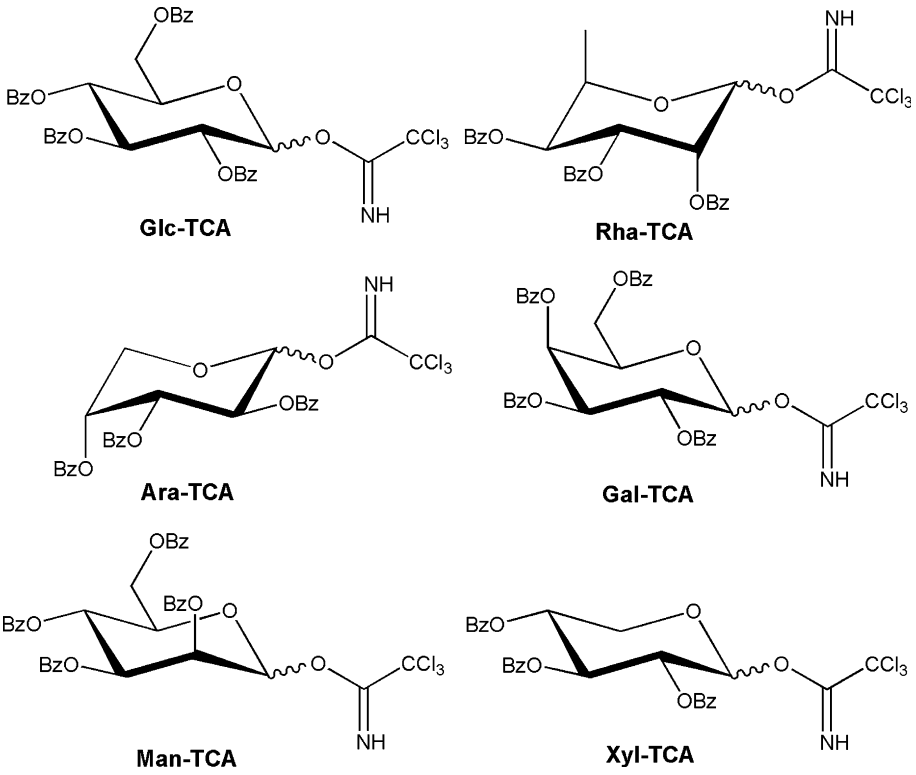


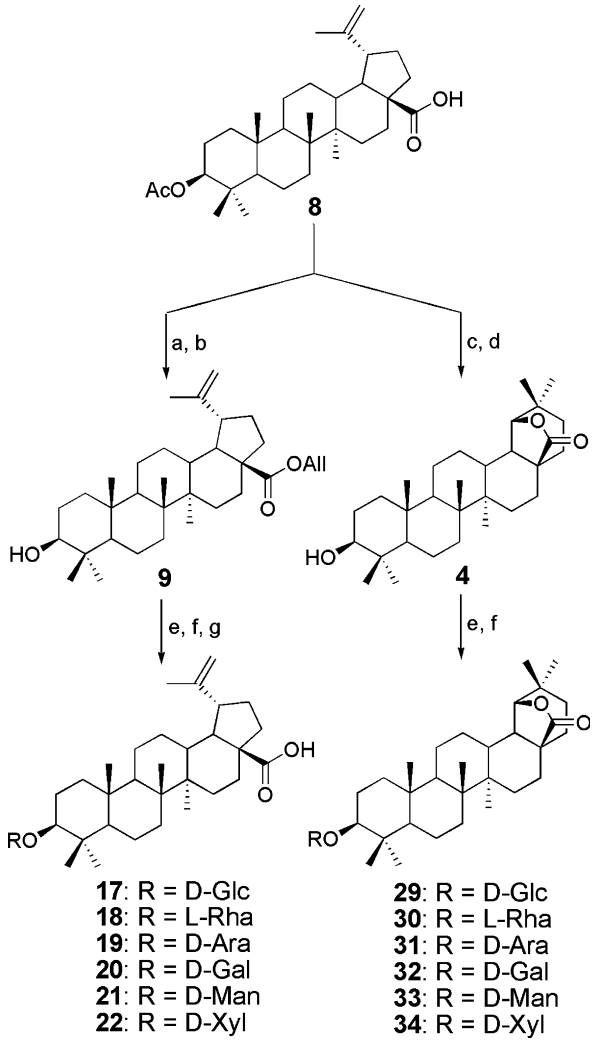
Figure 2. Trichloroacetimidate (TCA) sugar donors used for the glycosidation.

anosides, β-D-xylopyranosides and α-D-mannopyranosides). This was confirmed by the chemical shifts and the vicinal coupling constants of the anomeric protons in ^{1}H NMR experiments.[52] The structures of all newly synthesized saponins (**14**–**16**, **20**–**22**, **23**–**34**) were confirmed and elucidated by extended 1D and 2D NMR experiments (^{1}H, ^{13}C, DEPT135, COSY, HSQC, HMBC), IR spectra and HRMS. Saponins **16**[39d] and **18**[39c] are naturally occurring products whose synthesis had not been reported in the literature until now.

2.2. Cytotoxic activity

The in vitro antiproliferative activity of these triterpenes (**1**–**4**, **8**) and saponins (**11**–**34**) was investigated against human lung carcinoma (A-549) and human colorectal adenocarcinoma (DLD-1) cancer cell lines. We chose these types of cancer because they have a great incidence in the human population. Assessments were also carried out on normal human skin fibroblasts (WS1) to evaluate the differential selectivity of the target compounds. Cell lines cultured in essential medium were plated on 96-well microplates (5.0×10^3 cells per well) and allowed to adhere for 16 h before treatment at 37 °C in a humidified environment containing 5% CO_2. Thereafter, increasing concentrations of each compound in DMSO were added. The cell viability was assessed through the resazurin reduction test as previously described in the literature.[53] 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}). Betulinic acid (**2**) was used as a positive control in this experimentation (IC_{50} 10.3–15 μmol/L). Compounds with IC_{50} values >75 μmol/L were considered as inactive.

Our previous SAR investigations showed that adding a sugar moiety at the C-3 position of betulin (**1**) significantly decreased cytotoxicity compared to its carboxylic derivative **2**.[41] This extended study revealed that there are some exceptions to this general tendency. In fact, betulin 3β-*O*-α-D-mannopyranoside (**15**) was found to exhibit more potent cytotoxicity than betulinic acid (**2**) with IC_{50} values ranging from 7.3 to 10.1 μmol/L against cancer cell lines. In contrast to other sugars linked to the C-3 position of betulin (**1**) which suppress the anticancer activity, the specific stereochemistry of D-mannose seems to have a beneficial effect on the cytotoxicity of betulin (**1**). With regard to betulinic acid (**2**), we have previously shown that glycosidation at the C-3 position significantly increased both the activity and the selectivity towards cancer cell lines for D-glucose, L-rhamnose and D-arabinose monosaccharides.[41] In this study, betulinic acid 3β-*O*-α-D-mannopyranoside (**21**) and betulinic acid 3β-*O*-β-D-xylopyranoside (**22**) exerted moderate activity against A-549 (**21**: IC_{50} 34 μmol/L; **22**: IC_{50} 15 μmol/L) and DLD-1 (**21**: IC_{50} 15 μmol/L; **22:** IC_{50} 18 μmol/L) with cytotoxicity profiles slightly inferior to aglycone **2**, while the 3β-*O*-β-D-galactopyranoside **20** was inactive (IC_{50} > 75 μmol/L). Moreover, no significant increase in the selectivity was measured for these new betulinic acid (**2**) saponins. These results suggest that both the anticancer activity and the differential selectivity of betulinic acid (**2**) are strongly influenced by the nature of the carbohydrate moiety at the C-3 posi-



Scheme 3. Synthesis of betulinic acid (**17**–**22**) and 28-oxoallobetulin (**29**–**34**) saponins. Reagents and conditions: (a) allyl bromide (2 equiv), K_2CO_3 (3 equiv), DMF, 55 °C, 7 h; (b) NaOH 0.25 M, MeOH/THF/H_2O, rt, 2 h, 93% (two steps); (c) $FeCl_3/SiO_2$, DCM, reflux, 3 h; (d) NaOH 0.25 M, MeOH/THF/H_2O, reflux, overnight, 91% (two steps); (e) trichloroacetimidate (1.5 equiv), TMSOTf (0.05 equiv), 4 Å MS, DCM, rt, 1.5 h; (f) NaOH 0.25 M, MeOH/THF/H_2O, rt, 2 h; (g) $Pd(PPh_3)_4$ (0.3 equiv), PPh_3 (0.6 equiv), pyrrolidine (2 equiv), THF, rt, 1–3 d.

tion. It is interesting to note that the 3β-*O*-acetyl derivative of betulinic acid (**8**) was significantly less cytotoxic against human normal fibroblasts than against cancerous cell lines.

It appeared that modifications in the parent structure of lupane-type triterpenoids such as the loss of the isopropylene group at the C-20 position and the expansion of ring E to six carbons seem to have a deleterious effect on the cytotoxicity profile. Indeed, no significant diminution of the cancerous cells' growth was observed at the maximum tested concentration for both allobetulin (**3**) and 28-oxoallobetulin (**4**) (IC_{50} > 75 μmol/L). These results are in good agreement with those of Kim et al.[54] since they conclude that the C-20 isopropylene group of betulinic acid (**2**) is not a favourable position to derive in order to enhance the cytotoxicity. We thought that glycosidation could have a significant impact on the in vitro anticancer activity of these rare members of the germanicane-type triterpenoid family. Interestingly, 3β-*O*-β-D-glucopyranoside and 3β-*O*-β-D-galactopyranoside of allobetulin (**23**, **26**) exhibited moderate cytotoxicity towards cancer cell lines with IC_{50} values ranging from 30 to 40 μmol/L. It should be noted that, contrary to betulinic acid (**2**), the introduction of these sugar moieties at the C-3 position of **3** resulted in an enhancement of the activity. Furthermore, all 3β-*O*-glycosides of 28-oxoallobetulin (**29**–**34**) were ineffective to inhibit the growth of the cancerous cell lines (IC_{50} > 75 μmol/L). In terms of SAR, these in vitro cytotoxic results suggest that lupane-type triterpenoid saponins are generally stronger anticancer agents than germanicane-type triterpenoid saponins and particularly those with betulinic acid (**2**) as aglycone.

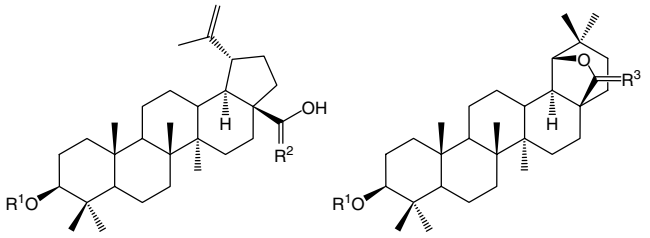
2.3. Predicted lipophilicity and aqueous solubility

In order to pass through biological membranes (gastrointestinal membrane, blood–brain barrier, cellular membrane, etc.), a therapeutic agent must be relatively soluble in water. In medicinal chemistry, the aqueous solubility and lipophilicity of a drug have proven to be important molecular parameters determining the absorption, the bioavailability and, sometimes, the bioactivity.[55] The aqueous solubility ($\log S$) reflects the concentration S of the compound in mol/L for a saturated aqueous solution in equilibrium with the crystalline material, while the lipophilicity is indicated by the logarithm of a partition coefficient ($\log P$) which reflects the concentration ratio of the drug at equilibrium partitioning between octanol and water phases.[42] In this study, we have used the ADME prediction program QikProp version 2.5 to evaluate in silico these parameters (Table 1). As we expected, adding a sugar moiety enhances the aqueous solubility (>$\log S$) and decreases the lipophilicity (<$\log P$) of lupane- and germanicane-type triterpenoids (**1**–**4**). These theoretical results are in good agreement with our experimental results since glycosides were shown to be more soluble in polar solvents (DMSO, MeOH) used for in vitro bioassays than their corresponding aglycones (data not shown). Interestingly, all synthesized glycosides (**11**–**34**) have $\log P$ values smaller than 5. Thus, concerning the partition coefficient, these compounds satisfy one of the 'Lipinski Rule-of-5' which is important to ensure a good intestinal absorption and permeation of the therapeutic agent when orally delivered.[56] Moreover, if we compare $\log P$ values of the most active betulinic acid (**2**) glycosides, we observe that the order of anticancer activity potential (**18** > **19** > **22** > **21**) generally follows the order of lipophilicity (**18** > **19** > **22** > **21**). Nevertheless, it is obvious that lipophilicity and aqueous solubility alone cannot be invoked to explain the difference in cytotoxicity of lupane- and germanicane-type triterpenoid saponins.

3. Conclusion

In summary, starting from betulin (**1**) isolated from the outer bark of *B. papyrifera*, we successfully synthesized

Table 1. In vitro cytotoxicity of lupane and germanicane triterpenes (**1**–**4**, **8**) and saponins (**11**–**34**)



Compound	R^1	R^2	R^3	Cell line IC_{50}[a] (μmol/L ± SD)			log P[e]	log S[f]
				A-549[b]	DLD-1[c]	WS1[d]		
1	H	H_2	—	3.80 ± 0.09[g]	6.6 ± 0.3[g]	3.58 ± 0.07[g]	6.06	−6.70
2	H	O	—	10.3 ± 0.4[g]	15.0 ± 0.3[g]	12 ± 1[g]	6.31	−6.91
3	H	—	H_2	>75	>75	>75	6.24	−7.44
4	H	—	O	>75	>75	>75	5.55	−6.83
8	Ac	O	—	18 ± 2[h]	20 ± 2[h]	57 ± 6	7.16	−8.30
11	Glc	H_2	—	>75[g]	>75[e]	> 75[e]	3.80	−6.09
12	Rha	H_2	—	22 ± 3[g]	50 ± 10[g]	33 ± 5[g]	4.57	−6.32
13	Ara	H_2	—	41 ± 3[g]	63 ± 8[g]	59 ± 5[g]	4.43	−5.94
14	Gal	H_2	—	>75	>75	>75	3.86	−6.04
15	Man	H_2	—	7.3 ± 0.4	10.1 ± 0.5	5.1 ± 0.6	4.08	−6.27
16	Xyl	H_2	—	>75	>75	>75	4.44	−6.64
17	Glc	O	—	>75[e]	32 ± 9[g,h]	> 75[g]	4.03	−6.33
18	Rha	O	—	2.6 ± 0.6[g,h]	3.9 ± 0.4[g,h]	31 ± 3[g]	4.81	−6.62
19	Ara	O	—	10 ± 2[g,h]	17 ± 3[g,h]	47 ± 5[g]	4.76	−6.80
20	Gal	O	—	>75	>75	>75	4.15	−6.13
21	Man	O	—	34 ± 4	15 ± 1	13 ± 3	4.08	−6.27
22	Xyl	O	—	15 ± 2	18 ± 2	20 ± 1	4.66	−6.75
23	Glc	—	H_2	31 ± 3	40 ± 10	40 ± 4	3.95	−6.61
24	Rha	—	H_2	>75	>75	>75	4.71	−6.94
25	Ara	—	H_2	>75	>75	>75	4.73	−6.97
26	Gal	—	H_2	30 ± 10	40 ± 10	30 ± 10	4.17	−6.30
27	Man	—	H_2	>75	>75	>75	3.99	−6.67
28	Xyl	—	H_2	>75	>75	>75	4.67	−6.96
29	Glc	—	O	>75	>75	>75	3.32	−6.31
30	Rha	—	O	>75	>75	>75	4.09	−6.56
31	Ara	—	O	>75	>75	>75	4.00	−6.64
32	Gal	—	O	>75	>75	>75	3.40	−6.29
33	Man	—	O	>75	>75	>75	3.34	−6.16
34	Xyl	—	O	>75	>75	>75	3.92	−6.57

Glc, β-D-glucopyranose; Rha, α-L-rhamnopyranose; Ara, α-D-arabinopyranose; Gal, β-D-galactopyranose; Man, α-D-mannopyranose; Xyl, β-D-xylopyranose; Ac, acetate.

[a] Data represent mean values ± standard deviation for three independent experiments made in triplicate.

[b] Human lung carcinoma.

[c] Human colorectal adenocarcinoma.

[d] Human normal skin fibroblasts.

[e] Predicted octanol/water partition coefficient.

[f] Predicted aqueous solubility.

[g] Results previously reported.[41]

[h] Significantly more sensitive than fibroblasts; $p < 0.05$, Student's t test.

a library of 3β-*O*-monodesmosidic saponins (**11**–**34**) having betulin (**1**), betulinic acid (**2**), allobetulin (**3**) and 28-oxoallobetulin (**4**) as aglycones, and β-D-glucopyranose, α-L-rhamnopyranose, α-D-arabinopyranose, β-D-galactopyranose, α-D-mannopyranose and β-D-xylopyranose as sugar moieties, in order to enhance the cytotoxicity and the hydrosolubility of germanicane- and lupane-type triterpenes. Saponins **16** and **18** are naturally occurring products whose synthesis had not been reported in the literature until now. This extended SAR study confirmed that saponins of betulinic acid (**17**–**22**) are generally better in vitro anticancer agents than those derived from betulin (**11**–**16**) with the exception of betulin 3β-*O*-α-D-mannopyranoside (**15**) which exhibited a strongly potent cytotoxic activity against cancerous cell lines. Furthermore, although the synthesis of novel germanicane-type saponins was carried out with success, the bioactivity measured for these glycosides was not as high as we anticipated since only 3β-*O*-β-D-glucopyranoside and 3β-*O*-β-D-galactopyranoside of allobetulin (**23**, **26**) showed moderate anticancer activity.

4. Experimental

4.1. Chemicals

Chemical reagents were purchased from Sigma–Aldrich Co. Canada or Alfa Aesar Co. and were used as received. The usual solvents were obtained from VWR International Co. and were used as received. Dichloromethane (DCM) was distilled from anhydrous CaH_2 under an argon atmosphere. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under an argon atmosphere. All anhydrous and air sensitive reactions were performed in oven-dried glassware under positive argon pressure. Tetrakistriphenylphosphine palladium(0) was prepared as mentioned in the literature[57] and stored under argon. Analytical thin-layer chromatography was performed with silica gel 60 F_{254}, 0.25 mm pre-coated TLC plates (Silicycle, Québec, Canada). Aliphatic compounds were visualised using aqueous sulfuric acid solution of ammonium heptamolybdate tetrahydrate (10 g/100 mL H_2SO_4 + 900 mL H_2O) and aromatic compounds were visualised using 254 nm and/or 365 nm UV wavelength. Flash column chromatographies were performed on 230–400 mesh silica gel R12030B (Silicycle, Québec, Canada). Infrared spectra (IR) were recorded with a Perkin-Elmer Spectrum One FT-IR spectrophotometer (Shelton, Connecticut, USA) on a NaCl window from a thin film of the analyzed compounds and only significant absorption bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance spectrometer at 400 MHz (^1H) and 100 MHz (^{13}C), equipped with a 5 mm QNP probe. Elucidations of chemical structures were based on ^1H, ^{13}C, COSY, HMBC, HSQC and DEPT-135 experiments. Signals are reported as m (multiplet), s (singlet), d (doublet), dd (doublet of doublet), ddt (doublet of doublet of triplet), br s (broad singlet) and coupling constants are reported in hertz (Hz). The chemical shifts are reported in ppm (δ) relative to residual solvent peak. The labile OH NMR signals appearing sometimes were not listed. Optical rotations were obtained in a solution of 10% MeOH in $CHCl_3$ (v/v) unless otherwise specified using sodium D line at ambient temperature on a Jasco DIP360 digital polarimeter. High-resolution mass spectra (HRMS) were obtained at the Department of Chemistry, Queen's University, Ontario, Canada. HPLC preparative methods for the purification of saponins **20**, **21** and **22** in DMSO (10 mg/mL) were carried out with an Agilent 1100 system equipped with a fraction collector using Zorbax ODS C18 column (21.2 × 250 mm; 7 μmol/L), at a flow rate of 16.0 mL/min with a column temperature of 25 °C. Compounds were detected by UV absorption at 254 nm following this elution gradient: 70–100% B where B = MeOH and A = H_2O + 0.1% HCOOH in 30 min (increasing concentration of B by 1%/min).

Sugar donors 2,3,4,6-tetra-*O*-benzoyl-α,β-D-glucopyranose trichloroacetimidate, 2,3,4-tri-*O*-benzoyl-α,β-L-rhamnopyranose trichloroacetimidate and 2,3,4-tri-*O*-benzoyl-α,β-D-arabinopyranose trichloroacetimidate were synthesized from D-glucose, L-rhamnose, and D-arabinose, respectively, following a procedure previously reported by our laboratory.[41] 2,3,4,5-Tetra-*O*-benzoyl-α,β-D-galactopyranose trichloroacetimidate,[58] 2,3,4,6-tetra-*O*-benzoyl-α,β-D-mannopyranose trichloroacetimidate[59] and 2,3,4-tri-*O*-benzoyl-α,β-D-xylopyranose trichloroacetimidate[49] were prepared from D-galactose, D-mannose and D-xylose, respectively, as previously reported. Crude betulin (**1**) was extracted from the outer bark of *B. papyrifera* March. and recrystallized with an azeotropic mixture of 2-butanol/water (74:26) to afford **1** with an acceptable purity (>95%) according to GC–MS. The synthesis of lupane-type saponins (**11**–**13** and **17**–**19**) was already reported by our laboratory.[41]

4.2. Betulin 3,28-diacetate (5)

To a solution of betulin (**1**) (2.33 g, 5.26 mmol) dissolved in 20 mL of pyridine at room temperature was added 1.33 mL of Ac_2O (14.10 mmol) followed by 64 mg of DMAP (0.53 mmol) and stirred for 1 h. Fifty millilitres of CH_2Cl_2 and 50 mL of distilled water were then poured into the solution, the mixture was acidified with H_2SO_4 6.2 M (25 mL) at 0 °C and then extracted three times with CH_2Cl_2 (25 mL). The combined organic phases were washed with saturated $NaHCO_3$ until pH ≈7. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Simple filtration over silica gel using EtOAc/hexanes (2.5:97.5) as eluent afforded 2.77 g of compound **5** as a white solid in a quantitative yield: mp 216–218 °C, lit.[60] mp 223–224 °C; $[\alpha]_D^{20}$ +19.7 (*c* 1.67, $CHCl_3$); IR: 2945, 2872, 1738 (C=O), 1641, 1455, 1365, 1243 (C–O ester), 1030, 979, 883, 738. ^1H and ^{13}C NMR spectral data of **5** were in agreement with those published in the literature.[61]

4.3. Betulin 3-acetate (6)

Compound **5** (250 mg, 0.470 mmol) and Al(*i*-OPr)$_3$ (490 mg, 2.35 mmol) were stirred under reflux in 8 mL of *i*-PrOH for 2 h. The crude mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (25 mL) and water (25 mL). The mixture was then extracted with CH_2Cl_2 (3× 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc/hexanes (10:90 to 20:80) as eluent to afford 0.20 g (86% yield) of compound **6** as a white solid: mp 258–260 °C, lit.[60] mp 256–258 °C; $[\alpha]_D^{20}$ +25.7 (*c* 0.92, $CHCl_3$); IR: 3438 (broad, OH), 2944, 2872, 1732 (C=O), 1454, 1374, 1245 (C–O ester), 1105, 1028 (C–OH), 979, 882, 738. ^1H and ^{13}C NMR spectral data of **6** were in agreement with those published in the literature.[62]

4.4. 3-Acetylbetulinal (7)

Compound **6** (9.77 g, 20.2 mmol) and PCC (8.73 g, 40.3 mmol) were added to 980 mL of CH_2Cl_2 and stirred at room temperature for 2 h. Silica gel (30–50 g) was poured into the mixture and the solvent was evaporated under reduced pressure to dryness. The residual dry-pack was directly used for purification by flash chromatography on silica gel using EtOAc/hexanes (5:95) as

eluent to afford 9.34 g (96% yield) of aldehyde **7** as a white solid: $[\alpha]_D^{20}$ +31.7° (*c* 0.71, $CHC1_3$), lit.[60] $[\alpha]_D^{20}$+30.3 (*c* 2.0, $CHCl_3$); IR: 2943, 1731 (C═O), 1641, 1451, 1375, 1244 (C–O ester), 1027, 979, 883, 738. ^{1}H NMR ($CDCl_3$): 9.66 (s, 1H), 4.75 (br s, 1H, H-29), 4.62 (br s, 1H, H-29), 4.45 (dd, 1H, *J* = 9.9 Hz, *J* = 5.8 Hz, H-3), 2.86 (m, 1H), 2.20–0.70 (24H), 2.03 (s, 3H), 1.69 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.83 (s, 6H), 0.82 (s, 3H); ^{13}C NMR ($CDCl_3$): 206.85, 171.19, 149.85, 110.34, 81.04, 59.47, 55.52, 50.51, 48.17, 47.69, 42.69, 40.97, 38.81, 38.54, 37.92, 37.22, 34.38, 33.35, 29.98, 29.36, 38.93, 28.07, 25.62, 23.82, 21.46, 20.89, 19.11, 18.28, 16.62, 16.34, 16.03, 14.36; HR-EI-MS *m*/*z* 482.3779 $[M]^+$ (calcd for $C_{32}H_{50}O_3$, 482.3760).

4.5. 3-Acetylbetulinic acid (8)

Compound **7** (1.00 g, 2.27 mmol) was dissolved in 50 mL of *t*-BuOH, 10 mL of distilled THF and 15 mL of 2-methyl-2-butene. The solution was stirred and cooled in an ice-bath. Then, 30 mL of freshly prepared solution of aqueous NaH_2PO_4/$NaClO_2$ (2.50 g/2.50 g in 30 mL of distilled water) was slowly added to the solution and the mixture was stirred for 15 min. The mixture was then raised to rt and stirred for 1 h. Finally, the mixture was poured into 50 mL of a saturated solution of NH_4Cl and extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. Purification of the crude product by flash chromatography using isocratic 7% ethyl acetate in hexanes as eluent afforded 772 mg (81% yield) of carboxylic acid **8** as a white solid: $[\alpha]_D^{20}$ +26.4° (*c* 0.54, $CHCl_3$); IR: 2945, 1735 (C═O), 1696 (C═O), 1452, 1369, 1244 (C–O ester), 1027, 979. ^{1}H NMR ($CDCl_3$): 4.74 (br s, 1H, H-29), 4.61 (br s, 1H, H-29), 4.47 (dd, 1H, *J* = 10.4 Hz, *J* = 5.6 Hz, H-3), 3.00 (m, 1H), 2.30–0.70 (25H), 2.04 (s, 3H), 1.69 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H); ^{13}C NMR ($CDCl_3$): 182.19, 171.21, 150.51, 109.90, 81.09, 56.54, 55.55, 50.53, 49.40, 47.09, 42.56, 40.83, 38.56, 38.52, 37.95, 37.27, 37.19, 34.37, 32.30, 30.71, 29.84, 28.10, 25.58, 23.84, 21.48, 20.99, 19.50, 18.31, 16.62, 16.33, 16.19, 14.81; HR-EI-MS *m*/*z* 521.3629 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_4Na$, 521.3607).

4.6. Allyl betulinate (9)

Compound **8** (1.00 g, 2.01 mmol) was solubilized with 14 mL of anhydrous dimethylformamide (DMF) and the solution was stirred at 55 °C. Then, 831 mg of anhydrous K_2CO_3 (6.03 mmol) and 339 μL of allyl bromide (4.02 mmol) where added to the mixture and stirred at this temperature for 7 h. After cooling, CH_2Cl_2 was added and the organic layer was washed twice with HCl 10% and once with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude 3-acetoxy-28-allyl betulinate was dissolved in a 1:2:1 MeOH/THF/H_2O (100 mL) solution containing NaOH (≈0.25 N) and stirred for 3 h (TLC monitored). After, 50 mL of CH_2Cl_2 was added and the solution was neutralized with HCl 10% until pH 4–5 and extracted with CH_2Cl_2 three times with portions of 50 mL. Combined organic layers were dried over $Na_2S_2O_4$, filtered and evaporated under reduced pressure. Crude product was purified by flash chromatography on silica gel using EtOAc/hexanes (5:95 to 17.5:82.5) as eluent to afford 922 mg (93% yield for two steps) of compound **9** as a white solid: mp 152–154 °C; $[\alpha]_D^{20}$ +3.9 (*c* 1.00, $CHCl_3$); IR: 3421, 2945, 2869, 1725, 1642, 1452, 1376, 1270, 1152, 1131, 1106, 1043, 982, 884, 738. ^{1}H and ^{13}C NMR spectral data of **9** were in agreement with those previously reported by our laboratory.[41]

4.7. Betulin 28-acetate (10)

Compound **1** (1.00 g, 2.07 mmol) and DMAP (28 mg, 0.21 mmol) were dissolved in 15 mL of pyridine and stirred at room temperature. Then, 0.21 mL of Ac_2O (2.07 mmol) was added dropwise and stirred for 1 h. After, the solution was diluted with 50 mL of CH_2Cl_2 and poured into a separatory funnel filled with ice and 40 mL of H_2SO_4 6.2 M was slowly added. The aqueous phase was extracted three times with CH_2Cl_2 (50 mL) and combined organic phases were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc/hexanes (5:95 to 20:80) as eluent. Betulin 28-acetate (**10**) (0.80 g, 73% yield) was obtained with betulin 3,28-diacetate (**5**) (0.13 g, 11% yield) and starting betulin (**1**) (0.13 g, 13% yield), all as white solids: mp 210–212 °C; $[\alpha]_D^{20}$ +8.5 (*c* 1.58, $CHCl_3$); IR: 3468 (broad, OH), 2942, 2870, 1739 (C═O), 1641, 1454, 1388, 1364, 1237 (C–O ester), 1033 (C–OH), 981, 883, 738. ^{1}H and ^{13}C NMR spectral data of **10** were in agreement with those published in the literature.[61]

4.8. Allobetulin (3)

This compound was prepared as previously reported[16] following this procedure: 5.00 g of betulin (**1**) (11.29 mmol), dissolved in 500 mL of CH_2Cl_2 with a mixture of $Fe(NO_3)_3$/SiO_2 (1:4) grounded on a mortar (9.13 g/36.50 g, 22.58 mmol of $Fe(NO_3)_3$) were refluxed for 45 min. The solution was then filtered and washed with CH_2Cl_2 and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc/hexanes (10:90 to 20:80) as eluent to afford 3.60 g (72% yield) of allobetulin (**3**) as a white solid: $[\alpha]_D^{20}$ +44.5° (*c* 0.65, $CHCl_3$); IR: 3452, 2926, 2863, 1450, 1386, 1264, 1180, 1138, 1088, 1042, 1005, 987, 971, 887, 810, 768, 737. ^{1}H NMR ($CDCl_3$): 3.76 (d, 1H, *J* = 7.6 Hz, H-28), 3.52 (s, 1H, H-19), 3.43 (d, 1H, *J* = 7.8 Hz, H-28), 3.19 (m, 1H, H-3), 2.00–1.00 (24H), 0.96 (s, 6H), 0.92 (s, 3H), 0.90 (s, 3H), 0.83 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H); ^{13}C NMR ($CDCl_3$): 88.06, 79.08, 71.39, 55.60, 51.20, 46.95, 41.60, 40.83, 40.73, 39.04, 39.01, 37.38, 36.87, 36.39, 34.26, 34.03, 32.83, 28.94, 28.11, 27.54, 26.58, 26.57, 26.39, 24.68, 21.11, 18.38, 16.62, 15.84, 15.52, 13.64; HR-EI-MS *m*/*z* 443.3905 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_2Na$, 443.3889).

4.9. 28-Oxoallobetulin (4)

Carboxylic acid **8** (500 mg, 1.00 mmol), was stirred under reflux in 25 mL of CH_2Cl_2 with a mixture of

$FeCl_3/SiO_2$ (1:4) grounded on a mortar (0.50 g/1.95 g, 3.00 mmol of $FeCl_3$) for 3 h. The mixture was then filtered on celite and washed with CH_2Cl_2, evaporated and dissolved in a 1:2:1 MeOH/THF/H_2O (50 mL) solution and was refluxed with 1.00 g of NaOH (25 mmol) overnight. Then, 25 mL of CH_2Cl_2 was added and the solution was neutralized with HCl 10% until pH 4–5 and extracted with CH_2Cl_2 three times with portions of 50 mL. Combined organic layers were dried over $Na_2S_2O_4$, filtered and evaporated, affording crude product which was purified by flash chromatography on silica gel with MeOH/CH_2Cl_2 (1:99 to 3:97) as eluent to give 417 mg (91% yield over two steps) of compound **4** as a white solid: $[\alpha]_D^{20}$ +51.7° (*c* 0.49, $CHCl_3$); IR: 3377, 2941, 1760, 1446, 1388, 1153, 1119, 1045, 966, 922, 733. ^{1}H NMR ($CDCl_3$): 3.93 (s, 1H, H-19), 3.20 (dd, 1H, J = 11.2 Hz, J = 4.9 Hz, H-3), 2.00–0.50 (24H), 1.02 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.90 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H), 0.75 (s, 3H); ^{13}C NMR ($CDCl_3$): 179.86, 85.99, 78.89, 55.49, 51.23, 46.70, 46.09, 40.55, 39.91, 38.93, 38.87, 37.25, 36.00, 33.71, 33.54, 32.31, 31.93, 28.74, 27.94, 27.88, 27.35, 26.51, 25.54, 23.95, 20.87, 18.14, 16.53, 15.51, 15.34, 13.65; HR-EI-MS *m*/*z* 457.3696 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 457.3682).

4.10. General procedure for the synthesis of glycosides 11–22 and 29–34

One equivalent of the appropriate acceptor and 1.5 equivalents of the appropriate donor were dissolved in dry CH_2Cl_2 (30 mM) with 4 Å molecular sieves and stirred at room temperature. TMSOTf (0.05 equiv) was then added dropwise under an argon atmosphere while keeping rigorous anhydrous conditions. The reaction was usually performed in approximately 1.5 h and then neutralized by adding triethylamine (8 M). The crude mixture was filtered and evaporated under reduced pressure. The residue was dissolved in a NaOH 0.25 N solution of MeOH/THF/H_2O (0.01 M) and stirred at rt for 2 h. The mixture was diluted in CH_2Cl_2 and washed with HCl 10% and brine. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel using 2–10% MeOH in CH_2Cl_2 as eluent.

4.11. General procedure for the synthesis of glycosides 23–28

One equivalent of the appropriate acceptor and 1.5 equivalents of the appropriate donor were solubilized in dry CH_2Cl_2 (30 mM) with 4 Å molecular sieves and stirred at room temperature. TMSOTf (0.05 equiv) was then added dropwise under an argon atmosphere while keeping rigorous anhydrous conditions. The reaction was usually performed in approximately 1.5 h and then neutralized by adding triethylamine (8 M). The crude mixture was filtered and evaporated under reduced pressure. The residue was dissolved in a NaOH 0.25 N solution of MeOH/THF/H_2O (0.01 M) and stirred at room temperature for 2 h. The mixture was diluted in CH_2Cl_2 and washed with HCl 10% and brine. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was dissolved in a solution of triphenylphosphine (PPh_3) (0.6 equiv) and pyrrolidine (2 equiv) in dry THF (0.22 M), then $Pd^0(PPh_3)_4$ (0.3 equiv) was added and the resulting mixture was stirred for 1–3 days at room temperature under argon atmosphere. After evaporation of the solvent under reduced pressure, the residue was purified by flash chromatography using 2–20% MeOH in CH_2Cl_2 as eluent.

4.12. Betulin 3β-*O*-β-D-galactopyranoside (14)

This compound was prepared from **10** (250 mg, 0.52 mmol) to afford 60 mg of **14** (19% yield) as a white solid: $[\alpha]_D^{20}$ +9.5° (*c* 0.60); IR: 3373, 2920, 2853, 1457, 1353, 1246, 1145, 1029, 973, 876. ^{1}H NMR (Pyr-d_5): 4.90 (m, 2H, H-1′, H-29), 4.75 (s, 1H, H-29), 4.62 (s, 1H, H-4′), 4.51 (m, 3H, H-6′ (2x), H-2′), 4.20 (m, 1H, H-3′), 4.16 (m, 1H, H-5′), 4.12 (m, 1H, H-28), 3.68 (m, 1H, H-28), 3.43 (m, 1H, H-3) 2.70–0.60 (25H), 1.78 (s, 3H), 1.33 (s, 3H), 1.10 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (Pyr-d_5): 151.64, 110.33, 107.98, 89.14, 77.25, 75.91, 73.60, 70.72, 62.89, 59.82, 56.24, 51.02, 49.51, 48.94, 48.73, 43.37, 41.57, 40.05, 39.45, 37.95, 37.46, 35.26, 34.99, 30.78, 30.39, 28.52, 27.94, 27.27, 26.11, 21.45, 19.66, 18.87, 17.20, 16.75, 16.50, 15.33; HR-ESI-MS *m*/*z* 627.4214 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4237).

4.13. Betulin 3β-*O*-α-D-mannopyranoside (15)

This compound was prepared from **10** (261 mg, 0.54 mmol) to afford 159 mg of **15** (49% yield) as a white powder: $[\alpha]_D^{20}$ +57.5° (*c* 0.58); IR: 3303, 2933, 2866, 1451, 1374, 1058, 1056, 978, 880, 679. ^{1}H NMR (Pyr-d_5): 5.61 (br s, 1H, H-1′), 4.90 (d, 1H, J = 2.2 Hz, H-29), 4.76 (s, 1H, H-29), 4.73 (m, 1H, H-4′), 4.64 (m, 1H, H-3′), 4.62 (m, 1H, H-6′), 4.57 (m, 1H, H-2′), 4.51 (m, 1H, H-5′), 4.45 (m, 1H, H-6′), 4.09 (d, 1H, J = 11.2 Hz, H-28), 3.67 (d, 1H, J = 10.7 Hz, H-28), 3.52 (dd, 1H, J = 11.5 Hz, J = 4.2 Hz, H-3), 2.70–0.60 (25H), 1.78 (s, 3H), 1.16 (s, 3H), 1.02 (s, 3H), 0.96 (s, 3H), 0.84 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (Pyr-d_5): 151.65, 110.33, 98.12, 81.99, 76.39, 73.63, 73.40, 69.61, 63.80, 59.82, 56.17, 50.94, 49.49, 48.92, 48.72, 43.34, 41.54, 39.10, 38.81, 37.93, 37.62, 35.25, 34.90, 30.77, 30.40, 29.27, 27.92, 26.05, 22.60, 21.42, 19.66, 18.88, 17.15, 16.67, 16.50, 15.33; HR-ESI-MS *m*/*z* 627.4243 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4237).

4.14. Betulin 3β-*O*-β-D-xylopyranoside (16)

This compound was prepared from **10** (251 mg, 0.52 mmol) to afford 81 mg of **16** (27% yield) as a white solid: $[\alpha]_D^{20}$ +2.4 ° (*c* 0.38); IR: 3343, 2937, 2866, 1450, 1374, 1242, 1161, 1039, 974, 880, 635. ^{1}H NMR (Pyr-d_5): 4.90 (d, 1H, J = 2.1 Hz, H-29), 4.88 (d, 1H, J = 7.6 Hz, H-1′), 4.75 (s, 1H, H-29), 4.40 (m, 1H, H-5′), 4.26 (m, 1H, H-4′), 4.19 (m, 1H, H-3′), 4.11 (d, 1H, J = 10.6 Hz, H-28), 4.06 (m, 1H, H-2′), 3.80 (m, 1H, H-5′), 3.68 (d, 1H, J = 10.4 Hz, H-28), 3.41 (dd, 1H, J = 11.7 Hz, J = 4.4 Hz, H-3), 2.70–0.70 (25H),

1.77 (s, 3H), 1.33 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (Pyr-d_5): 151.06, 110.35, 108.08, 89.07, 79.04, 75.97, 71.64, 67.54, 59.76, 56.24, 51.03, 49.50, 48.93, 48.72, 43.35, 41.58, 40.10, 39.41, 37.94, 37.51, 35.26, 34.96, 30.76, 30.41, 28.49, 27.94, 27.35, 26.06, 21.43, 19.64, 18.86, 17.20, 16.76, 16.51, 15.29; HR-ESI-MS *m/z* 597.4146 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_6Na$, 597.4131).

4.15. Allobetulin 3β-*O*-β-D-glucopyranoside (23)

This compound was prepared from **3** (80 mg, 0.18 mmol) to afford 82 mg of **23** (75% yield) as a white solid: $[\alpha]_D^{20}$ +20.3° (*c* 0.57); IR: 3350, 2923, 2865, 1448, 1387, 1374, 1358, 1304, 1162, 1072, 1035, 1022, 893, 766. ^{1}H NMR (Pyr-d_5): 4.98 (d, 1H, *J* = 7.8 Hz, H-1′), 4.64 (m, 1H, H-6′), 4.45 (m, 1H, H-6′), 4.26 (m, 2H, H-3′ and H-4′), 4.07 (m, 1H, H-2′), 4.04 (m, 1H, H-5′), 3.87 (d, 1H, *J* = 7.8 Hz, H-28), 3.68 (s, 1H, H-19), 3.51 (d, 1H, *J* = 7.6 Hz, H-28), 3.41 (m, 1H, H-3), 2,28 (m, 1H, H-2), 1.87 (m, 1H, H-2), 1.70–0.70 (22H), 1.34 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.97 (s, 3H), 0.88 (s, 3H), 0.84 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (Pyr-d_5): 107.36, 89.21, 88.23, 79.18, 78.77, 76.21, 72.27, 71.63, 63.48, 56.37, 51.63, 47.52, 42.02, 41.31, 41.18, 40.04, 39.49, 37.51, 37.32, 36.92, 34.89, 34.61, 33.55, 29.60, 28.51, 27.18, 27.15, 27.11, 26.93, 24.97, 21.67, 18.79, 17.25, 17.07, 16.18, 14.05; HR-ESI-MS *m/z* 627.4220 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4237).

4.16. Allobetulin 3β-*O*-α-L-rhamnopyranoside (24)

This compound was prepared from **3** (100 mg, 0.23 mmol) to afford 110 mg of **24** (83% yield) as a white solid: $[\alpha]_D^{20}$ −3.3° (*c* 0.27); IR: 3408, 2926, 1448, 1386, 1130, 1106, 1051, 974, 811. ^{1}H NMR (Pyr-d_5): 5.36 (d, 1H, *J* = 1.2 Hz, H-1′), 4.61 (m, 1H, H-2′), 4.50 (m, 1H, H-3′), 4.36 (m, 1H, H-5′), 4.34 (m, 1H, H-4′), 3.87 (d, 1H, *J* = 7.4 Hz, H-28), 3.68 (s, 1H, H-19), 3.51 (d, 1H, *J* = 7.8 Hz, H-28), 2.00 (m, 1H, H-2), 1.90–0.60 (23H), 1.71 (d, 3H, *J* = 5.7 Hz, H-6′), 1.09 (s, 3H), 0.94 (s, 3H), 0.94 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (Pyr-d_5): 104.89, 88.87, 88.22, 74.52, 73.33, 72.90, 71.62, 70.25, 56.11, 51.60, 47.50, 42.00, 41.29, 41.15, 39.70, 39.32, 37.49, 37.30, 36.91, 34.88, 34.53, 33.54, 29.58, 28.50, 27.13, 27.09, 26.90, 26.44, 24.96, 21.67, 18.92, 18.87, 17.01, 16.94, 16.16, 14.01; HR-ESI-MS *m/z* 611.4267 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_6Na$, 611.4288).

4.17. Allobetulin 3β-*O*-α-D-arabinopyranoside (25)

This compound was prepared from **3** (100 mg, 0.23 mmol) to afford 103 mg of **25** (79% yield) as a white solid: $[\alpha]_D^{20}$ +51.9° (*c* 0.47); IR: 3343, 2939, 2926, 2871, 2855, 1450, 1386, 1337, 1290, 1252, 1069, 1033, 1001, 939, 767, 714. ^{1}H NMR (Pyr-d_5): 4.76 (d, 1H, *J* = 7.1 Hz, H-1′), 4.46 (m, 1H, H-2′), 4.41 (m, 1H, H-5′), 4.38 (m, 1H, H-4′), 4.23 (m, 1H, H-3′), 3.88 (m, 1H, H-5′), 3.85 (d, 1H, *J* = 6.8 Hz, H-28), 3.69 (s, 1H, H-19), 3.51 (d, 1H, *J* = 7.7 Hz, H-28), 3.46 (dd, 1H, *J* = 12.4 Hz, *J* = 4.6 Hz, H-3), 2.03 (m, 1H, H-2), 1.80–0.60 (24H), 1.22 (s, 3H), 1.08 (s, 3H), 0.96 (s, 3H), 0.88 (s, 6H), 0.85 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (Pyr-d_5): 103.39, 88.21, 85.19, 75.21, 72.96, 71.63, 70.03, 67.49, 56.74, 51.61, 47.51, 42.00, 41.30, 41.19, 39.26, 39.01, 37.69, 37.30, 36.90, 34.85, 34.58, 33.54, 29.57, 28.90, 27.12, 27.09, 26.90, 24.95, 24.16, 21.68, 18.96, 17.29, 16.95, 16.16, 14.00; HR-ESI-MS *m/z* 597.4130 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_6Na$, 597.4131).

4.18. Allobetulin 3β-*O*-β-D-galactopyranoside (26)

This compound was prepared from **3** (100 mg, 0.23 mmol) to afford 91 mg of **26** (67% yield) as a white solid: $[\alpha]_D^{20}$ +21.6° (*c* 0.41); IR: 3407, 2941, 2868, 1641, 1449, 1386, 1140, 1056, 978, 667. ^{1}H NMR (Pyr-d_5): 4.92, (d, 1H, *J* = 7.8 Hz, H-1′), 4.63 (d, 1H, *J* = 3.0 Hz, H-4′), 4.53 (m, 2H, H-6′), 4.50 (m, 1H, H-2′), 4.22 (m, 1H, H-3′), 4.17 (m, 1H, H-5′), 3.87 (d, 1H, *J* = 8.0 Hz, H-28), 3.68 (s, 1H, H-19), 3.51 (d, 1H, *J* = 7.8 Hz, H-28), 3.41 (m, 1H, H-3), 2.32 (m, 1H, H-2), 1.92 (m, 1H, H-2), 1.70–0.70 (22H), 1.33 (s, 3H), 1.09 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (Pyr-d_5): 107.57, 88.67, 87.82, 76.87, 75.48, 73.18, 71.22, 70.30, 62.49, 55.97, 51.23, 47.11, 41.60, 40.89, 40.77, 39.65, 39.12, 37.10, 36.90, 36.50, 34.47, 34.19, 33.14, 29.17, 28.09, 26.86, 26.72, 26.70, 26.50, 24.54, 21.25, 18.36, 16.79, 16.66, 15.76, 13.63; HR-ESI-MS *m/z* 627.4215 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4237).

4.19. Allobetulin 3β-*O*-α-D-mannopyranoside (27)

This compound was prepared from **3** (100 mg, 0.23 mmol) to afford 121 mg of **27** (89% yield) as a white solid: $[\alpha]_D^{20}$ +79.8° (*c* 0.54); IR: 3364, 2924, 2868, 1443, 1386, 1123, 1069, 1033, 811, 713. ^{1}H NMR (Pyr-d_5): 5.62 (d, 1H, *J* = 1.2 Hz, H-1′), 4.76 (m, 1H, H-4′), 4.65 (m, 1H, H-3′), 4.63 (m, 1H, H-6′), 4.59 (m, 1H, H-2′), 4.50 (m, 1H, H-5′), 4.48 (m, 1H, H-6′), 3.87 (d, 1H, *J* = 7.8 Hz, H-28), 3.68 (s, 1H, H-19), 3.51 (d, 1H, *J* = 7.6 Hz, H-28), 3.51 (m, 1H, H-3), 1.84 (m, 1H, H-2), 1.70–0.70 (23H), 1.18, (s, 3H), 1.08 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (Pyr-d_5): 98.09, 88.21, 81.85, 76.43, 73.67, 73.42, 71.62, 69.60, 63.81, 56.33, 51.55, 47.51, 42.02, 41.29, 41.16, 39.13, 38.88, 37.68, 37.30, 36.91, 34.85, 34.51, 33.54, 29.58, 29.26, 27.13, 27.08, 26.89, 24.95, 22.55, 21.65, 18.82, 17.17, 17.00, 16.16, 14.05; HR-ESI-MS *m/z* 627.4221 $[M+Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.4237).

4.20. Allobetulin 3β-*O*-β-D-xylopyranoside (28)

This compound was prepared from **3** (100 mg, 0.23 mmol) to afford 110 mg of **28** (85% yield) as a white solid: $[\alpha]_D^{20}$ +17.7° (*c* 0.57); IR: 3250, 2923, 1441, 1385, 1165, 1086, 1032, 969, 892, 767. ^{1}H NMR (Pyr-d_5): 4.88 (d, 1H, *J* = 7.6 Hz, H-1′), 4.43, (m, 1H, H-5′), 4.29 (m, 1H, H-4′), 4.22 (m, 1H, H-3′), 4.07 (m, 1H, H-2′), 3.87 (d, 1H, *J* = 8.2 Hz, H-28), 3.82 (m, 1H, H-5′), 3.68 (s, 1H, H-19), 3.52 (d, 1H, *J* = 8.0 Hz, H-28), 3.38 (m, 1H, H-3), 2.24 (m, 1H, H-2), 1.95 (m, 1H, H-2), 1.70–0.70 (22H), 1.33 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 0.96 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H), 0.79 (s,

3H); ^{13}C NMR (Pyr-d_5): 108.13, 89.01, 88.21, 79.06, 75.98, 71.65, 71.63, 67.56, 56.40, 51.66, 47.51, 42.00, 41.30, 41.18, 40.12, 39.54, 37.56, 37.30, 36.91, 34.86, 34.60, 33.54, 29.58, 28.45, 27.35, 27.13, 27.09, 26.91, 24.96, 21.67, 18.78, 17.19, 17.09, 16.18, 14.01; HR-ESI-MS *m/z* 597.4144 $[M+Na]^+$ (calcd for $C_{35}H_{58}O_6$, 597.4131).

4.21. Betulinic acid 3β-*O*-β-D-galactopyranoside (20)

This compound was prepared from **9** (207 mg, 0.42 mmol) to afford 111 mg of **20** (43% yield) as a white solid: $[\alpha]_D^{20}$ −3.9° (*c* 0.20); IR: 3325, 2936, 2864, 1687, 1449, 1375, 1214, 1152, 1056, 976, 879. ^{1}H NMR (Pyr-d_5): 4.96 (s, 1H, H-29), 4.90 (d, 1H, *J* = 7.6 Hz, H-1′), 4.77 (s, 1H, H-29), 4.63 (m, 1H, H-4′), 4.50 (m, 3H, H-6′ (2x), H-2′), 4.21 (m, 1H, H-3′), 4.15 (m, 1H, H-5′), 3.56 (m, 1H, H-19), 3.42 (m, 1H, H-3) 2.80–0.60 (24H), 1.80 (s, 3H), 1.32 (s, 3H), 1.12 (s, 3H), 1.03 (s, 3H), 0.96 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.32, 151.69, 110.35, 107.95, 89.11, 77.25, 75.91, 73.62, 70.66, 62.84, 57.02, 56.31, 51.20, 50.13, 48.16, 43.22, 41.45, 40.04, 39.44, 38.96, 37.98, 37.50, 35.13, 33.27, 31.59, 30.65, 28.51, 27.26, 26.46, 21.56, 19.84, 18.83, 17.18, 16.76, 16.74, 15.25; HR-ESI-MS *m/z* 641.4005 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.22. Betulinic acid 3β-*O*-α-D-mannopyranoside (21)

This compound was prepared from **9** (201 mg, 0.40 mmol) to afford 58 mg of **21** (23% yield) as a white solid: $[\alpha]_D^{20}$ +71.6° (*c* 0.08); IR: 3382, 2944, 1686, 1440, 1376, 1241, 1106, 1058, 1028, 975, 881, 814. ^{1}H NMR (Pyr-d_5): 5.60 (br s, 1H, H-1′), 4.96 (br s, 1H, H-29), 4.78 (br s, 1H, H-29), 4.75 (m, 2H, H-4′), 4.63 (m, 2H, H-3′, H-6′), 4.57 (br s, 1H, H-2′), 4.49 (m, 2H, H-5′, H-6′), 3.55 (m, 1H, H-19), 3.53 (m, 1H, H-3), 3.00–0.50 (24H), 1.80 (s, 3H), 1.16 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.81 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.29, 151.73, 110.35, 98.10, 81.95, 76.41, 73.67, 73.41, 69.63, 63.82, 57.01, 56.24, 51.12, 50.11, 50.06, 43.19, 41.43, 39.09, 38.92, 38.81, 37.98, 37.66, 35.04, 33.24, 31.57, 30.62, 29.26, 26.42, 22.56, 21.52, 19.82, 18.87, 17.13, 16.74, 16.65, 15.25; HR-ESI-MS *m/z* 641.4017 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.23. Betulinic acid 3β-*O*-β-D-xylopyranoside (22)

This compound was prepared from **9** (200 mg, 0.40 mmol) to afford 138 mg of **22** (58% yield) as a white solid: $[\alpha]_D^{20}$ +11.9° (*c* 0.15); IR: 3376, 2931, 2865, 1687, 1638, 1453, 1375, 1161, 1046, 974, 882. ^{1}H NMR (Pyr-d_5): 4.96 (s, 1H, H-29), 4.87 (d, 1H, *J* = 7.0 Hz, H-1′), 4.78 (s, 1H, H-29), 4.39 (m, 1H, H-5′), 4.26 (m, 1H, H-4′), 4.20 (m, 1H, H-3′), 4.05 (m, 1H, H-2′), 3.80 (m, 1H, H-5′), 3.56 (m, 1H, H-19), 3.40 (m, 1H, H-3), 2.80–0.70 (24H), 1.79 (s, 3H), 1.32 (s, 3H), 1.11 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.27, 151.67, 110.39, 108.09, 89.06, 79.05, 75.98, 71.64, 67.54, 57.01, 56.31, 51.20, 50.12, 48.16, 43.20, 41.46, 40.09, 39.45, 38.94, 37.97, 37.56, 35.11, 33.24, 31.57, 30.64, 28.48, 27.35, 26.44, 21.56, 19.81, 18.85, 17.18, 16.75 (2x), 15.22; HR-ESI-MS *m/z* 587.3961 $[M-H]^-$ (calcd for $C_{35}H_{55}O_7$, 587.3953).

4.24. 28-Oxoallobetulin 3β-*O*-β-D-glucopyranoside (29)

This compound was prepared from **4** (80 mg, 0.18 mmol) to afford 56 mg of **29** (50% yield) as a white solid: $[\alpha]_D^{20}$ +23.1° (*c* 0.41); IR: 3388, 2943, 2869, 1766, 1447, 1388, 1375, 1304, 1154, 1072, 1016, 969, 923, 532. ^{1}H NMR (Pyr-d_5): 4.98 (d, 1H, *J* = 7.8 Hz, H-1′), 4.64 (m, 1H, H-6′), 4.45 (m, 1H, H-6′), 4.26 (m, 2H, H-3′, H-4′), 4.08 (m, 1H, H-2′), 4.06 (s, 1H, H-19), 4.04 (m, 1H, H-5′), 3.40 (m, 1H, H-3), 2.28 (m, 1H, H-2), 2.00 (m, 1H, H-16), 1.86 (m, 2H, H-2, H-18), 1.70–0.70 (20H), 1.32 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.78 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.92, 107.36, 89.16, 86.25, 79.16, 78.77, 76.19, 72.25, 63.48, 56.33, 51.72, 47.18, 46.60, 41.10, 40.55, 40.00, 39.47, 37.46, 36.88, 34.40, 34.11, 33.12, 32.44, 29.19, 28.65, 28.47, 27.14, 26.97, 26.38, 24.05, 21.51, 18.66, 17.19, 17.07, 15.86, 14.12; HR-ESI-MS *m/z* 641.4038 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.25. 28-Oxoallobetulin 3β-*O*-α-L-rhamnopyranoside (30)

This compound was prepared from **4** (100 mg, 0.22 mmol) to afford 92 mg of **30** (70% yield) as a white solid: $[\alpha]_D^{20}$ −6.8° (*c* 0.27); IR: 3310, 2935, 1757, 1443, 1387, 1146, 1117, 1053, 965, 921, 810. ^{1}H NMR (Pyr-d_5): 5.36 (d, 1H, *J* = 1.2 Hz, H-1′), 4.62 (m, 1H, H-2′), 4.53 (m, 1H, H-3′), 4.37 (m, 1H, H-5′), 4.35 (m, 1H, H-4′), 4.07 (s, 1H, H-19), 3.17 (m, 1H, H-3), 2.00 (m, 1H, H-2), 2.00 (m, 1H, H-16), 1.87 (m, 1H, H-18), 1.80–0.60 (21H), 1.72 (d, 3H, *J* = 5.7 Hz, H-6′), 1.04 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.94, 104.94, 88.84, 86.26, 74.53, 73.35, 72.92, 70.28, 56.10, 51.72, 47.19, 46.59, 41.08, 40.56, 39.69, 39.31, 37.47, 36.88, 34.34, 34.12, 33.13, 32.45, 29.20, 28.66, 28.48, 26.97, 26.43, 26.39, 24.06, 21.55, 18.95, 18.77, 17.05, 16.91, 15.87, 14.10; HR-ESI-MS *m/z* 625.4055 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_7Na$, 625.4080).

4.26. 28-Oxoallobetulin 3β-*O*-α-D-arabinopyranoside (31)

This compound was prepared from **4** (250 mg, 0.10 mmol) to afford 26 mg of **31** (20% yield) as a white solid: $[\alpha]_D^{20}$ +50.9° (*c* 0.25); IR: 3280, 2941, 2921, 1757, 1442, 1386, 1360, 1137, 1068, 1002, 965, 945, 921. ^{1}H NMR (Pyr-d_5): 4.75 (d, 1H, *J* = 7.1 Hz, H-1′), 4.45 (m, 1H, H-2′), 4.41 (m, 1H, H-5′), 4.38 (m, 1H, H-4′), 4.23 (m, 1H, H-3′), 4.07 (s, 1H, H-19), 3.85 (d, 1H, *J* = 12.6 Hz, H-5′), 3.43 (m, 1H, H-3), 2.20–0.70 (24H), 1.24 (s, 3H), 1.03 (s, 3H), 0.93 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.78 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.95, 103.33, 86.23, 85.07, 75.20, 72.94, 70.01, 67.47, 56.71, 51.71, 47.18, 46.57, 41.10, 40.55, 39.24, 38.98, 37.65, 36.84, 34.38, 34.09, 33.10, 32.42, 29.16, 28.86, 28.63, 26.96, 26.37, 24.10, 24.03, 21.54, 18.84, 17.23, 16.96, 15.85, 14.06; HR-ESI-MS *m/z* 611.3935 $[M+Na]^+$ (calcd for $C_{35}H_{56}O_7Na$, 611.3924).

4.27. 28-Oxoallobetulin 3β-*O*-β-D-galactopyranoside (32)

This compound was prepared from **4** (100 mg, 0.22 mmol) to afford 83 mg of **32** (61% yield) as a white solid: $[\alpha]_D^{20}$ +28.0° (*c* 0.47); IR: 3378, 2935, 1758, 1446, 1389, 1153, 1055, 966, 922, 756. ^{1}H NMR (Pyr-d_5): 4.91, (d, 1H, *J* = 7.7 Hz, H-1′), 4.63 (d, 1H, *J* = 3.0 Hz, H-4′), 4.53 (m, 2H, H-6′), 4.51 (m, 1H, H-2′), 4.22 (m, 1H, H-3′), 4.17 (m, 1H, H-5′), 4.07 (s, 1H, H-19), 3.40 (m, 1H, H-3), 2.32 (m, 1H, H-2), 2.01 (m, 1H, H-16), 1.90 (m, 1H, H-2), 1.88 (m, 1H, H-18), 1.70–0.70 (20H), 1.32 (s, 3H), 1.04 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.90 (s, 3H), 0.78 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.94, 107.99, 89.02, 86.25, 77.29, 75.88, 73.58, 70.72, 62.91, 56.36, 51.75, 47.18, 46.59, 41.10, 40.56, 40.04, 39.52, 37.49, 36.88, 34.41, 34.12, 33.13, 32.44, 29.19, 28.66, 28.47, 27.24, 26.99, 26.39, 24.05, 21.53, 18.67, 17.16, 17.10, 15.87, 14.12; HR-ESI-MS *m*/*z* 641.4037 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.28. 28-Oxoallobetulin 3β-*O*-α-D-mannopyranoside (33)

This compound was prepared from **4** (100 mg, 0.22 mmol) to afford 62 mg of **33** (46% yield) as a white solid: $[\alpha]_D^{20}$ +83.1° (*c* 0.30); IR: 3330, 2940, 1757, 1443, 1388, 1119, 1067, 965, 921. ^{1}H NMR (Pyr-d_5): 5.62 (d, 1H, *J* = 1.1 Hz, H-1′), 4.76 (m, 1H, H-4′), 4.65 (m, 1H, H-3′), 4.63 (m, 1H, H-6′), 4.59 (m, 1H, H-2′), 4.50 (m, 1H, H-5′), 4.48 (m, 1H, H-6′), 4.06 (s, 1H, H-19), 3.51 (m, 1H, H-3), 1.99 (m, 1H, H-16), 1.86 (m, 1H, H-18), 1.84 (m, 1H, H-2), 1.70–0.70 (21H), 1.16 (s, 3H), 1.02 (s, 3H), 0.92 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H), 0.77 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.99, 98.05, 86.23, 81.72, 76.44, 73.67, 73.42, 69.62, 63.84, 56.29, 51.66, 47.17, 46.59, 41.07, 40.54, 39.15, 38.87, 37.65, 36.84, 34.32, 34.11, 33.12, 32.43, 29.23, 29.18, 28.65, 26.95, 26.36, 24.04, 22.50, 21.51, 18.71, 17.12, 17.02, 15.85, 14.13; HR-ESI-MS *m*/*z* 641.4043 $[M+Na]^+$ (calcd for $C_{36}H_{58}O_8Na$, 641.4029).

4.29. 28-Oxoallobetulin 3β-*O*-β-D-xylopyranoside (34)

This compound was prepared from **4** (100 mg, 0.22 mmol) to afford 28 mg of **34** (22% yield) as a white solid: $[\alpha]_D^{20}$ +37.3° (*c* 0.07); IR: 3230, 2922, 2853, 1757, 1443, 1386, 1260, 1166, 1044, 966, 921, 712. ^{1}H NMR (Pyr-d_5): 4.88 (d, 1H, *J* = 7.4 Hz, H-1′), 4.43 (m, 1H, H-5′), 4.28 (m, 1H, H-4′), 4.22 (m, 1H, H-3′), 4.07 (m, 1H, H-2′), 4.06 (s, 1H, H-19), 3.82 (m, 1H, H-5′), 3.37 (m, 1H, H-3), 2.24 (m, 1H, H-2), 1.95 (m, 1H, H-2), 1.80–0.70 (24 H), 1.32 (s, 3H), 1.03 (s, 3H), 1.00 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.79 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (Pyr-d_5): 179.95, 108.16, 88.94, 86.24, 79.08, 75.98, 71.65, 67.57, 56.37, 51.76, 47.18, 46.58, 41.10, 40.55, 40.10, 39.52, 37.53, 36.85, 34.40, 34.10, 33.11, 32.44, 29.18, 28.65, 28.41, 27.32, 26.96, 26.38, 24.05, 21.53, 18.67, 17.13, 17.11, 15.86, 14.08; HR-ESI-MS *m*/*z* 611.3914 $[M+Na]^+$ (calcd for $C_{35}H_{56}O_7Na$, 611.3924).

4.30. Cell lines and culture conditions

Human lung carcinoma (A-549), human colon adenocarcinoma (DLD-1) and human normal fibroblast (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% 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.

4.31. 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[53] 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.

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