EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201402187

Synthesis of Lupane-Type Saponins Containing an Unusual α-D-Idopyranoside Fragment as Potent Cytotoxic Agents

Piotr Cmoch,[a] Anna Korda,[a] Lucie Rárová,[b] Jana Oklešťková,[b] Miroslav Strnad,*[b] Katarzyna Gwardiak,[a] Romuald Karczewski,[a] and Zbigniew Pakulski*[a]

Dedicated to Professor Mieczysław Mąkosza on the occasion of his 80th birthday

Keywords: Carbohydrates / Glycoconjugates / Glycosylation / Steroids / Saponins / Drug discovery

A practical method for the preparation of benzoyl protected allyl and benzyl α-D-idopyranosides, and D-idopyranosyl trichloroacetimidate, from 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose, is described. All derivatives can be prepared on a multigram scale and require only simple chromatographic purification. A concise synthesis of lupane triterpenes bearing an unusual D-idopyranoside fragment is described. All new compounds were evaluated in vitro for their cytotoxic activities. Novel saponins exhibited interesting cytotoxic activity in the micromolar range against human cancer cell lines including T-lymphoblastic leukemia CEM, breast adenocarcinoma MCF7, and cervical carcinoma HeLa, but also against normal human fibroblasts BJ.

1. Introduction

Saponins are steroid or triterpenoid glycosides that are widely distributed in plants and in some marine organisms.[1,2] They possess interesting biological properties including antitumor, antiviral, antifungal, and antiinflammatory activities, which have been extensively studied and reviewed.[3–10] Their biological effects have been correlated to both sugar residues and aglycones.[3,11–13] Usually, mono-, di-, tri-, or tetrasaccharides are attached to the sapogenin backbone, where they constitutes a hydrophilic part, whereas sapogenin forms a hydrophobic fragment. Natural saponins based on a betulin scaffold occur less frequently than those having other triterpene-type aglycones, although growing interest in their synthesis is noticeable.[14–26] Further investigations are required to address questions regarding structure–activity relationships (SAR) and the role of the saccharide part. The high importance of L-idose derivatives in biological systems[27,28] and growing interest in D-idose derivatives, which occur very rarely in nature,[29–31] prompted us to prepare a series of lupane saponins containing a D-idopyranoside ring.

The chemistry of L-idose and L-idopyranuronic acid is well known, and numerous syntheses of these carbohydrates have been published because of the significant role played by these sugars as components of some glycosaminoglycans (e.g., heparin).[27,28] In comparison, D-idose has not been isolated from natural sources, although it is present in the capsular polysaccharide (CPS) structure of *Campylobacter jejuni* in the form of 6-deoxy-D-*ido*-heptose,[29,32] and was extracted from the unusual resurrection plant *Craterostigma plantagineum* as D-*glycero*-D-*ido*-2-octulose.[30,31] Chemical syntheses of D-idose and its derivatives, however, are extremely limited, and only a few examples have been reported. Paulsen reported a rearrangement of tetra-*O*-acetyl-D-glucopyranosyl chloride into α-D-idopyranose peracetate in the presence of antimony pentachloride.[33,34] Another example involved isomerization of the D-galactose part of lactose into D-idose by its selective sulfonylation, epoxide formation, followed by an opening of the epoxide ring, which finally afforded 4-*O*-β-D-idopyranosyl-D-glucopyranose.[35,36] Free D-idose was also prepared by an elongation of the D-xylose chain by the Nef reaction.[37] Recently, a stereoselective conversion of D-galactose into orthogonally protected β-D-idopyranosides was reported.[38] During the realization of this project, the syntheses of new derivatives of peracetylated D-idopyranoses and D-idopyranosides were published by Thiem.[39] However, the synthesis of simple benzoylated D-idopyranosyl trichloroacetimidate (used as glycosyl donor), or the corresponding D-idopyranosides, remains challenging.

Glycosylation of lupane triterpenes with acetylated glycosyl donors usually causes serious problems and, in

[a] Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warszawa, Poland
E-mail: zbigniew.pakulski@icho.edu.pl
http://www.icho.edu.pl/eng/
[b] Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University & Institute of Experimental Botany ASCR, Šlechtitelů 11, 78371 Olomouc, Czech Republic
E-mail: miroslav.strnad@upol.cz
http://www.ueb.cas.cz/en/

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201402187.

many cases, the desired products cannot be obtained. Acetyl migration from glycosyl donor to acceptor is usually observed as the main, or even as the only process. This problem was fully solved by application of benzoylated glycosyl donors in which migration of the benzoyl group does not occur.

Herein, we report on the synthesis of perbenzoylated D-idose derivatives, as well as novel lupane-type saponins bearing a D-idopyranoside moiety, and their anticancer properties determined by a cytotoxicity evaluation of these compounds. This is a continuation of our research on the synthesis and utility of saponins obtained from triterpenes isolated from white birch bark.[24–26] The synthetic strategy is directed toward glycosylation of 3-*O*-acetylbetulinic acid **1**, betulin derivatives **2**–**4**, lupeol (**5**), and 28-*O*-acetyl-*epi*-betulin (**6**) with D-idopyranosyl trichloroacetimidates **7**[39] and **8** (Figure 1). Subsequent saponification afforded free saponins. The cytotoxicities of these saponins were tested against a series of normal and cancer cell lines.

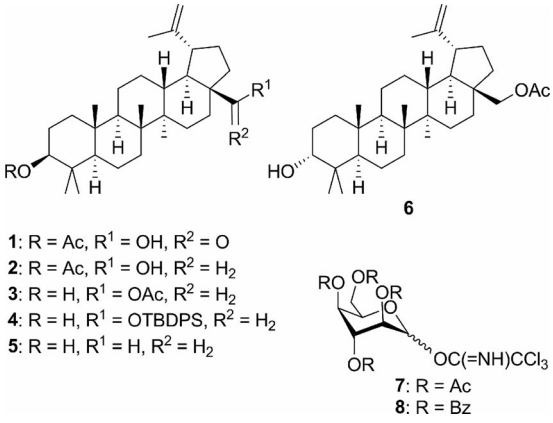
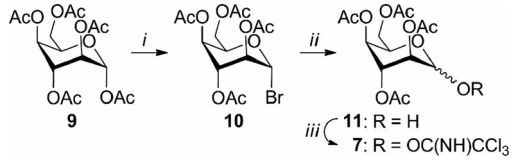


Figure 1. Parent triterpenes and glycosyl donors.

2. Results and Discussion

2.1. Synthesis of D-Idose Derivatives

The synthesis was initiated from α-D-idopyranose peracetate (**9**), which was easily prepared on a multigram scale according to the method reported by Paulsen.[33,34] Standard methods of hydrolysis of the anomeric acetate [hydrazine acetate[40] or ammonium carbonate in *N*,*N*-dimethylformamide (DMF)[41]] were unsuccessful and caused decomposition of the starting material. Therefore, the anomeric acetate was removed by using a two-step procedure (Scheme 1).[42] First, treatment of **9** with HBr in acetic acid gave glycosyl bromide **10**, which, in the second step, was converted into 2,3,4,6-tetra-*O*-acetyl-D-idopyranose (**11**) by hydrolysis induced by silver carbonate. The overall yield (86% after two steps) was slightly better than that obtained by using Thiem's method (treatment of **9** with $BnNH_2$, 80% yield).[39] Compound **11** was transformed in 69% yield into the required acetylated trichloroacetimidate **7** under the standard conditions (Scheme 1).

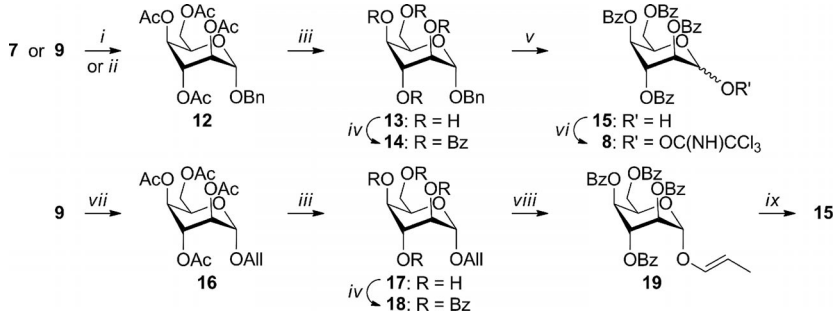


Scheme 1. Synthesis of acetylated D-idose derivatives. *Reagents and conditions:* (i) HBr in acetic acid; (ii) Ag_2CO_3, acetone/water; (iii) Cl_3CCN, K_2CO_3.

Reaction of **7** with benzyl alcohol in the presence of trimethylsilyl triflate (TMSOTf) gave benzyl α-D-idopyranoside **12** in 96% yield. Benzyl (**12**; 83%) and allyl α-D-idopyranosides (**16**; 72%) may be also prepared directly from peracetate **9** by treatment with benzyl or allyl alcohol, respectively, in the presence of $BF_3{\cdot}Et_2O$ complex.

Similar rearrangement of D-glucose perbenzoate into α-D-idopyranose perbenzoate is possible, but the product is formed in very low yield (12%).[43] Therefore, we decided to use idopyranosides **12** and **16** as starting materials for the preparation of the required benzoyl-protected D-idose derivatives. Deacetylation of **12** and **16** by methanolic sodium methoxide (to give **13** and **17**) followed by acylation with benzoyl chloride in pyridine afforded tetrabenzoates **14** and **18** in excellent yield (89% and 96%, respectively, after two steps).

Hydrogenolysis of benzyl idopyranoside **14** gave 2,3,4,6-tetra-*O*-benzoyl-D-idopyranose (**15**) in high yield (90%). The latter sugar was also prepared by hydrolysis (with HgO/$HgCl_2$;[44] 96%) of 1-propenyl α-D-idoside **19**, which was



Scheme 2. Synthesis of benzoylated D-idose derivatives. *Reagents and conditions:* (i) BnOH, $BF_3{\cdot}Et_2O$ (from **9**); (ii) BnOH, TMSOTf (from **7**); (iii) MeOH, MeONa; (iv) BzCl, pyridine; (v) H_2, 10%Pd/C; (vi) Cl_3CCN, K_2CO_3, DBU; (vii) AllOH, $BF_3{\cdot}Et_2O$; (viii) $[Ir(COD)(MePPh_2)_2]PF_6$; (ix) HgO/$HgCl_2$, acetone, water.

obtained in 98% yield by isomerization of allyl glycoside **18** with iridium complex $[Ir(COD)(MePh_2P)_2]PF_6$.[45] Transformation of **15** into the required donor **8** was achieved in 80% yield by treatment with trichloroacetonitrile in the presence of potassium carbonate and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU); the use of this mixture of both bases was crucial (Scheme 2).

2.2. Synthesis of Saponins

Glycosylation of 3-*O*-acetylbetulinic acid **1**[24] was performed by treatment with peracetylated (**7**) or perbenzoylated (**8**) D-idopyranosyl trichloroacetimidate in the presence of TMSOTf under standard conditions.[25] Protected esters were obtained in high yields (89% for **20** and 86% for **21**). Similar reaction of peracetylated donor **7** with lupeol (**5**)[25] and 28-*O*-acetylbetulin (**3**)[24] afforded acetylated triterpenes as the only products, as a result of acetyl migration from glycosyl donor to acceptor. Reactions of perbenzoylated donor **8** with lupeol (**5**) and 3-*O*-acetylbetulin (**2**)[24] afforded the expected α-D-idosides **23** (97%), and **25** (87%). When 28-*O*-acetylbetulin (**3**) was used as starting material, glycoside **27** was obtained in 81% yield. Reaction of *tert*-butyldiphenylsilyl protected betulin derivative **4**[46] with donor **8** gave α-D-idopyranoside **29** in 87% yield. Desilylation of **29** with acetic acid buffered tetrabutylammonium fluoride solution gave saponin **30** (78%). Because bidesmosidic saponins bearing sugar moieties at both C-3 and C-28 positions were considered to be less haemolytic than monodesmosides,[10,48] we investigated the synthesis of saponin **32**. Glycosylation of **30** with donor **8** afforded bidesmosidic saponin **32** in excellent yield (97%). Unexpectedly for us, reaction of 28-*O*-acetyl-*epi*-betulin (**6**)[47] with **8** gave the required product **34** in only 52% yield.

Final deprotection of the hydroxy groups was performed by treatment of benzoates with potassium carbonate in methanol, except for acetate protected **20**, which was treated with sodium methoxide in methanol. All saponins were prepared in good yields (60–83%) with the exception of bidesmosidic saponin **33**, which was isolated in 40% yield, probably due to the very poor solubility and high affinity to silica of the latter, which caused unusual loss of product (Figure 2). The structures of all synthesized saponins were confirmed by extensive 1D and 2D NMR analysis, as well as by elemental and HRMS analysis.

2.3. Discussion

It is widely accepted that 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose (**9**) exists mainly in $^4C_1$ or $^0S_2$ conformations.[49,50] In these forms, the H-1, H-2, H-3, and H-4 ring protons are in equatorial positions, which results in small coupling constants (2–3 Hz). In fact, all coupling constants observed in the ^{1}H NMR spectra of the studied compounds were small. This confirmed the D-ido configuration and the α-position of substituents at the anomeric center. Full analysis of the ^{1}H NMR spectra was, however, not trivial. Signals were poorly resolved and often occurred as complex multiplets caused by long-range couplings ($^4J_{1,3}$, $^5J_{1,4}$, and $^4J_{2,4}$), usually observed for idose derivatives.[49]

The coupling constants for the olefinic protons of the 1-propenyl part of **19** (12.3 Hz) confirmed the (*E*) configuration across the double bond. The (*Z*) isomer of **19** was not identified in the reaction mixture, although it may be present in a small amount (< 5%).

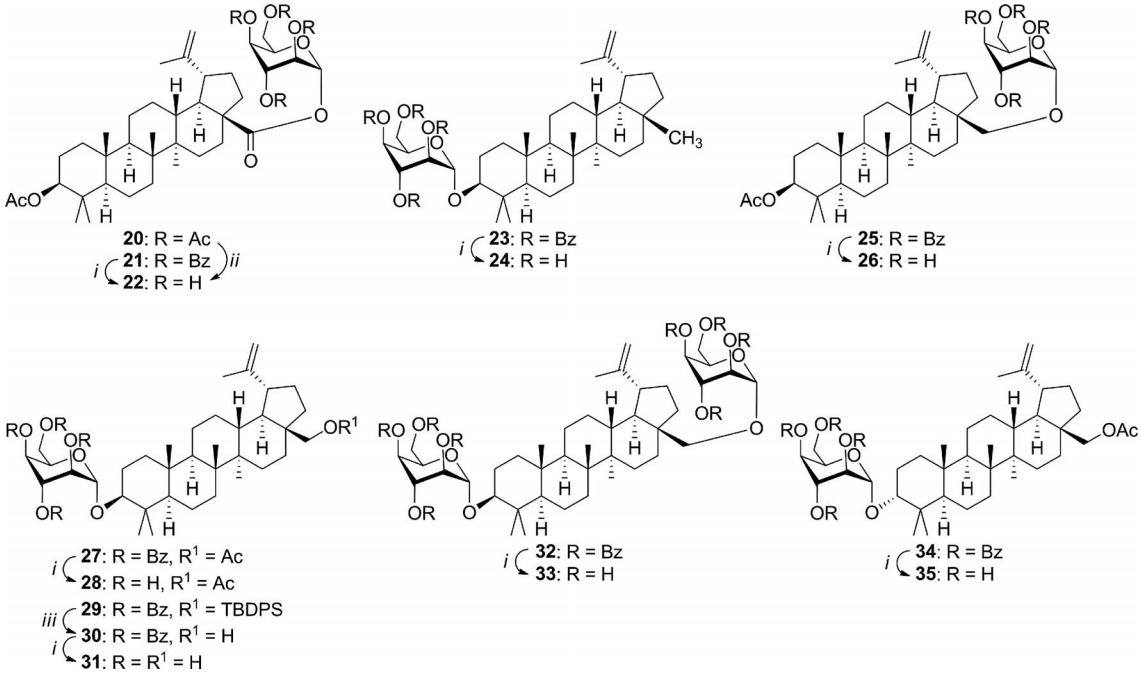
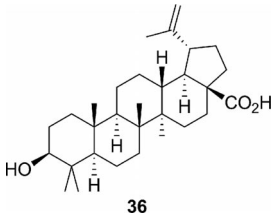


Figure 2. Synthesis of lupane saponins. *Reagents and conditions*: (i) K_2CO_3, MeOH; (ii) NaOMe, MeOH; (iii) Bu_4NF, AcOH, THF.

As expected, the presence of the acetyl and benzoyl protecting groups in the donor molecules directed the anomeric selectivity of the glycosidation reaction,[54] and in all cases studied in this report, 1,2-*trans*-idopyranosides were formed exclusively. This observation was strongly supported by $^1J_{C1,H1}$ coupling constants of 174 (in the case of **20**) and 170 Hz (in the case of **23**), which clearly indicated an equatorial proton at C-1.[51–53]

2.4. In Vitro Results

Anticancer activities of the lupane triterpenes bearing the unusual D-idopyranoside fragment were tested in vitro. Several normal and cancer cell lines were cultured and used to examine the structure–activity relationships of these lupane triterpenes. We compared the in vitro cytotoxic activity of selected analogues against human BJ fibroblasts and cancer cell lines of various histopathological origins, including T-lymphoblastic leukemia CEM, breast adenocarcinoma MCF7, and cervical carcinoma HeLa. Cells of all of these lines were exposed to six serial threefold dilutions of each drug for 72 h, the proportions of surviving cells were then estimated and IC_{50} values (50% inhibitory concentrations) were calculated by using betulinic acid (**36**; Figure 3) as a positive control. The results obtained from Calcein AM assays are presented in Table 1.



36

Figure 3. Betulinic acid (**36**).

Table 1. IC_{50} [μM] values obtained from the Calcein AM assays with the tested cancer and normal cells; mean±SD values obtained from three independent experiments performed in triplicate. Betulinic acid (**36**; Figure 3) was used as a positive control.

	CEM	MCF7	HeLa	BJ
22	25.5 ± 1.8	33.0 ± 4.9	19.4 ± 2.2	20.1 ± 2.5
24	38.6 ± 3.3	> 50	34.2 ± 4.0	16.1 ± 0.8
26	10.9 ± 1.6	17.6 ± 3.5	9.3 ± 1.0	7.3 ± 1.2
28	29.4 ± 1.6	37.6 ± 3.0	17.3 ± 2.5	13.9 ± 0.1
31	32.9 ± 3.4	47.2 ± 2.4	27.7 ± 4.1	42.1 ± 0.6
33	14.7 ± 1.0	30.1 ± 0.1	21.1 ± 7.0	21.2 ± 2.9
35	31.6 ± 1.6	33.7 ± 12.2	16.2 ± 1.5	9.9 ± 1.6
36	40.0 ± 2.8	> 50	47.6 ± 1.9	> 50

All tested lupane-type saponins bearing the D-idopyranoside moiety showed cytotoxic activity towards the cancer and normal cells used (except for **24** and **36** on MCF7 cells). Potent compounds were saponins **26**, **28**, and **35**, which showed cytotoxic activity against all of the tumor cell lines at low micromolar range (IC_{50} 9.3–37.6 μM) but usually also much stronger toxicity towards normal BJ fibroblasts (IC_{50} 7.3–20.1 μM). The therapeutic index of this series of lupane saponins containing a D-idopyranose ring is thus not very high because of their high cytotoxicity for normal human fibroblasts. The novel lupane-type saponins were, except for **24**, **33**, and **36**, also more potent for HeLa cell line, whereas bidesmosidic saponin **33** was the most active against CEM but less active than the parent compound **26**. The results show that several unique saponins bearing the D-idopyranose ring exhibit anticancer activities on several human cancer cell lines in the low micromolar range.

3. Conclusions

We have developed a practical method with which to obtain a series of D-idopyranose derivatives. Rearrangement of glucose peracetate, according to Paulsen and co-workers, provides rare monosaccharide D-idose. Simple manipulation with the protecting groups afforded series of acetyl and benzoyl protected derivatives, including allyl and benzyl glycosides, and the corresponding trichloroacetimidates, which are valuable glycosyl donors. This constitutes the first convenient method for the preparation of benzoyl protected D-idopyranose derivatives, which are especially useful in the synthesis of lupane-type saponins.

A series of the lupane-type saponins bearing the D-idopyranose ring were also synthesized and evaluated for their cytotoxic activities. Many of the studied compounds showed interesting cytotoxic activities in the low micromolar range against human cancer cell lines CEM, MCF7, and HeLa, but also comparable cytotoxicity on normal human fibroblasts (BJ).

4. Experimental Section

4.1. General Notes: Silica gel HF_{254} and silica gel 230–400 mesh (E. Merck) were used for TLC and column chromatography, respectively. ^1H and ^{13}C NMR spectra were recorded at 298 K with a Varian NMR-vnmrs600 or vnmrs500 spectrometers. Standard experimental conditions and standard Varian programs (ChemPack 4.1) were used with TMS as internal standard. Configurational assignments were based on the NMR measurements including two-dimensional techniques. High-resolution mass spectra (HRMS ESI) were acquired with MARINER and MaldiSYNAPT G2-S HDMS (Waters) mass spectrometers. Optical rotations were measured with a JASCO P-2000 automatic polarimeter. IR spectra were recorded with a Jasco 6200 FTIR spectrophotometer.

4.2. Syntheses

4.2.1. 2,3,4,6-Tetra-*O*-acetyl-α,β-D-idopyranose (11): 2,3,4,6-Tetra-*O*-acetyl-α-D-idopyranosyl bromide (**10**) was obtained by a modified literature method.[55] To a solution of 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose[33,34] (**9**; 11.50 g, 29.5 mmol) in 1,2-dichloroethane (100 mL), 33% HBr in acetic acid (30 mL) was added and the mixture was stirred at room temp. for 24 h. The mixture was diluted with 1,2-dichloroethane (100 mL) and the organic solution was washed with ice water (2× 100 mL), satd. $NaHCO_3$ (50 mL), cold water, dried with anhydrous sodium sulfate, and concentrated to afford crude 2,3,4,6-tetra-*O*-acetyl-α-D-idopyranosyl bromide (**10**; 11.00 g, 91%). Bromide **10** was dissolved in acetone (100 mL) and

water (3 mL), silver carbonate (8.50 g, 30.8 mmol) was added and the resulting suspension was stirred in the dark until disappearance of the starting material was confirmed by TLC (ca. 3 h). The mixture was filtered through a Celite pad, concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate, 7:3→1:1) to afford the title compound (8.81 g, 86% after two steps) with physicochemical properties identical to those reported.[39]

4.2.2. 2,3,4,6-Tetra-*O*-acetyl-α,β-D-idopyranosyl Trichloroacetimidate (7): To a solution of 2,3,4,6-tetra-*O*-acetyl-D-idopyranose (**11**; 4.00 g, 11.5 mmol) and trichloroacetonitrile (4.3 mL, 43.0 mmol) in dichloromethane (100 mL), potassium carbonate (3.90 g, 28.2 mmol) was added and the mixture was stirred at room temp. for 24 h. The mixture was then concentrated and the residue was purified by column chromatography (hexane/ethyl acetate, 20:1→1:1) to afford the title compound (α,β-mixture, 3.93 g, 69%) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (s, 1 H, NHα), 8.66 (s, 1 H, NHβ), 6.39 (d, $J_{1,2}$ = 2.9 Hz, 1 H, 1-Hβ), 6.28 (br. s, 1 H, 1-Hα), 5.48 (dd, $J_{3,2}$ = $J_{3,4}$ = 7.0 Hz, 1 H, 3-Hβ), 5.17 (dd, $J_{2,1}$ = 2.9, $J_{2,3}$ = 7.2 Hz, 1 H, 2-Hβ), 5.09–5.05 (m, 2 H, 3-Hα, 4-Hβ), 5.02–5.01 (m, 1 H, 2-Hα), 4.94–4.91 (m, 1 H, 4-Hα), 4.65–4.60 (m, 1 H, 5-Hα), 4.53–4.48 (m, 1 H, 5-Hβ), 4.38–4.35 (m, 2 H, 6-,6′-Hβ), 4.25 (dd, $J_{5,6}$ = 5.3, $J_{6,6'}$ = 11.6 Hz, 1 H, 6-Hα), 4.17 (dd, $J_{5,6'}$ = 7.3, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-Hα) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4 (α-C=O), 170.3 (β-C=O), 169.7 (α-C=O), 169.6 (β-C=O), 169.5 (β-C=O), 169.0 (β-C=O), 168.9 (α-C=O), 168.8 (α-C=O), 160.8 (β-C=NH), 159.9 (α-C=NH), 94.0 (C-1β), 93.9 (C-1α), 71.9 (β), 67.6 (β), 67.6 (β), 66.7 (β), 66.3 (α), 65.7 (α), 65.6 (α), 65.2 (α),62.8 (C-6β), 62.1 (C-6α) ppm. HRMS (ESI): *m*/*z* calcd. for $C_{16}H_{20}Cl_3NNaO_{10}$ $[M + Na]^+$ 514.0045; found 514.0032. $C_{16}H_{20}Cl_3NO_{10}$ (492.70): calcd. C 39.00, H 4.09, N 2.84, Cl 21.59; found C 38.83, H 4.17, N 2.86, Cl 21.40.

4.2.3. Benzyl 2,3,4,6-Tetra-*O*-acetyl-α-D-idopyranoside (12): *Method A*: A solution of trichloroacetimidate **7** (3.00 g, 6.1 mmol) and benzyl alcohol (0.76 mL, 7.34 mmol) in dichloromethane (60 mL) was stirred for 20–30 min at room temperature over molecular sieves (4 Å, 1.8 g, finely ground), then cooled to 0 °C and TMSOTf (360 μL, 2.00 mmol) was added and the mixture was stirred at 0 °C for 20 min. The reaction was quenched with Et_3N (2.0 mL), then the mixture was concentrated and the residue was purified by column chromatography (hexane/ethyl acetate, 10:1) to give the title compound (2.58 g, 96%) as a thick oil.

Method B: A solution of peracetate **9** (3.13 g, 8.00 mmol) and benzyl alcohol (3.10 mL, 30.0 mmol) in dichloromethane (60 mL) was stirred for 20–30 min at room temperature over molecular sieves (4 Å, 1.0 g, finely ground), then $BF_3{\cdot}Et_2O$ (3.8 mL, 30.0 mmol) was added and the mixture was stirred at room temperature for 4 h. The reaction was quenched with Et_3N (7.0 mL), then the mixture was concentrated and purified by column chromatography (hexane/ethyl acetate, 40:1→2:1) to yield the title compound (2.92 g, 83%) as a thick oil. $[\alpha]_D^{20}$ = 75.3 (*c* = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 1746, 1371, 1225, 1052 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36–7.30 (m, 5 H, ArH), 5.00–5.02 (m, 1 H, 3-H), 4.92–4.90 (m, 3 H, 1,2,4-H), 4.77 and 4.54 (ABq, *J* = 11.9 Hz, 2 H, PhC*H*$_2$), 4.50–4.46 (m, 1 H, 5-H), 4.20 (d, 2 H, 6,6′-H), 2.12 (s, 3 H, CH_3), 2.08 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 2.05 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4 (C=O), 169.7 (C=O), 169.2 (C=O), 169.1 (C=O), 136.9, 128.4, 127.9, 127.5, 96.7 (C-1), 69.2 (Ph*C*H$_2$), 67.2, 67.1, 66.8, 64.6, 62.4 (C-6), 20.8 (CH_3), 20.7 (CH_3), 20.7 (CH_3), 20.6 (CH_3) ppm. $C_{21}H_{26}O_{10}$ (438.44): calcd. C 57.53, H 5.98; found C 57.81, H 6.19.

4.2.4. Allyl 2,3,4,6-Tetra-*O*-acetyl-α-D-idopyranoside (16): Obtained as described for the synthesis of compound **12** (Method B). Peracetate **9** (781 mg, 2.00 mmol), allyl alcohol (0.70 mL, 10.0 mmol), and molecular sieves (4 Å, 500 mg, finely ground), in dichloromethane (20 mL) were treated with $BF_3{\cdot}Et_2O$ (1.3 mL, 10.0 mmol), and purified by column chromatography to afford the title compound (556 mg, 72%) as a thick oil; the physicochemical properties were identical to those reported.[39]

4.2.5. Benzyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-idopyranoside (14): To a solution of **12** (2.92 g, 6.66 mmol) in methanol (25 mL), sodium methoxide in methanol (0.45 M, 0.20 mL) was added and the mixture was stirred for 1 h. The mixture was concentrated and the residue (crude **13**) was dissolved in pyridine (40 mL). Benzoyl chloride (4.6 mL, 40.0 mmol) was slowly added and the mixture was stirred overnight. The excess of benzoyl chloride was decomposed by addition of methanol (10 mL), then solvents were coevoparated with toluene, and the residue was purified by column chromatography (hexane/ethyl acetate, 40:1→1:1) to afford the title compound (4.07 g, 89% after two steps) as a foam. $[\alpha]_D^{20}$ = 23.0 (*c* = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 1723, 1451, 1266, 1109, 1027, 709 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.15–7.18 (m, 25 H, ArH), 5.66–5.64 (m, 1 H, 3-H), 5.39–5.37 (m, 1 H, 4-H), 5.28–5.25 (m, 2 H, 1,2-H), 4.98–4.95 (m, 1 H, 5-H), 4.91 and 4.62 (ABq, *J* = 11.2 Hz, 2 H, PhC*H*$_2$), 4.72 (dd, $J_{6,5}$ = 8.0, $J_{6,6'}$ = 11.6 Hz, 1 H, 6-H), 4.55 (dd, $J_{6',5}$ = 4.5, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.1 (C=O), 164.5 (C=O), 133.5, 133.4, 133.3, 133.2, 130.2–127.9 (aromatic carbons), 97.0 (C-1), 69.7 (Ph*C*H$_2$), 67.4, 66.6 (2× *C*H), 64.6, 63.6 (C-6) ppm. $C_{41}H_{34}O_{10}$ (686.72): calcd. C 71.71, H 4.99; found C 71.74, H 5.10.

4.2.6. Allyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-idopyranoside (18): Obtained as described for the synthesis of **14**. Allyl 2,3,4,6-tetra-*O*-acetyl-α-D-idopyranoside (**16**; 528 mg, 1.36 mmol) was treated with sodium methoxide to give crude **17**, followed by benzoyl chloride (875 μL, 7.5 mmol) to obtain the title compound (835 mg, 96% after two steps) as a foam. $[\alpha]_D^{20}$ = 27.0 (*c* = 0.5, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.14–7.17 (m, 20 H, ArH), 6.00–5.91 (m, 1 H, allyl C*H*=), 5.65–5.63 (m, 1 H, 3-H), 5.40–5.38 (m, 1 H, 4-H), 5.27–5.32 (m, 1 H, allyl =C*H*$_2$), 5.24–5.22 (m, 1 H, 2-H), 5.18–5.22 (m, 2 H, 1-H, allyl =C*H*$_2$), 4.93–4.89 (m, 1 H, 5-H), 4.71 (dd, $J_{6,5}$ = 7.9, $J_{6,6'}$ = 11.6 Hz, 1 H, 6-H), 4.54 (dd, $J_{6',5}$ = 4.7, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.34–4.39 (m, 1 H, allyl OC*H*), 4.11–4.16 (m, 1 H, allyl OC*H*) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.1 (C=O), 164.6 (C=O), 133.5, 133.5, 133.4, 133.3, 130.1–128.2 (aromatic protons), 117.8 (allyl =*C*H$_2$), 96.8 (C-1), 68.7 (allyl O*C*H$_2$), 67.4, 66.8, 66.7, 64.5, 63.4 (C-6) ppm. $C_{37}H_{32}O_{10}$ (636.66): calcd. C 69.80, H 5.07; found C 69.87, H 5.07.

4.2.7. (*E*)-1-Propenyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-idopyranoside (19): To degassed THF (10 mL), $[Ir(COD)(MePPh_2)_2]PF_6$ (7 mg, 0.008 mmol) was added under an Ar atmosphere. The Ar in the system was replaced with H_2 and the suspension was stirred at room temperature for 15 min. Insoluble red crystals slowly dissolved and the clear solution became colorless. This solution was transferred into a solution of allyl α-D-idopyranoside **18** (632 mg, 1.00 mmol) in THF (20 mL), stirred at room temperature for 1 h, and then concentrated. The residue was purified by column chromatography (hexane/ethyl acetate, 40:1→5:1) to give the title compound (630 mg, 98%) as a foam. $[\alpha]_D^{20}$ = 17.8 (*c* = 0.2, chloroform). IR (film): $\tilde{\nu}_{max}$ = 1725, 1602, 1452, 1316, 1268, 1172, 1112, 1070, 1028, 757, 710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.17–7.17 (m, 20 H, ArH), 6.29 (dq, ^4J = 1.6, *J* = 12.3 Hz, 1 H, OC*H*=), 5.66–5.64 (m, 1 H, 3-H), 5.39 (br. s, 1 H, 4-H), 5.37 (br. s, 1 H, 1-H), 5.26–5.25 (m, 1 H, 2-H), 5.25–5.19 (dq, *J* = 12.3, 6.8 Hz, 1 H,

=C*H*-), 4.92–4.88 (m, 1 H, 5-H), 4.67 (dd, $J_{5,6}$ = 8.2, $J_{6,6'}$ = 11.6 Hz, 1 H, 6-H), 4.53 (dd, $J_{6',5}$ = 4.3, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 1.49 (dd, ^4J = 1.6, J = 6.8 Hz, 3 H, -CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.1 (C=O), 164.5 (C=O), 142.4 (=*C*H), 133.6, 133.4, 133.2, 130.1–128.2 (aromatic protons), 105.1 (=*C*H), 96.6 (C-1), 66.6, 66.6, 66.4, 64.9, 63.4 (C-6), 12.3 (*C*H_3) ppm. $C_{37}H_{32}O_{10}$ (636.66): calcd. C 69.80, H 5.07; found C 69.74, H 5.15.

4.2.8. 2,3,4,6-Tetra-*O*-benzoyl-α,β-D-idopyranose (15): *Method A*: To a solution of benzyl α-D-idopyranoside **14** (3.70 g, 5.39 mmol) in either ethyl acetate (75 mL) or THF (30 mL), 10% palladium on charcoal (300 mg) was added and the mixture was stirred under hydrogen atmosphere for 16 h. The mixture was filtered through a short silica pad, and purified by column chromatography (hexane/ethyl acetate, 10:1 → 1:1) to give recovered starting material (0.18 g, 5%), and the title compound (2.89 g, 90%) as a foam.

Method B: To a solution of **19** (600 mg, 0.94 mmol) in a mixture of acetone/water (10:1, 10 mL), yellow mercury oxide (270 mg, 1.25 mmol) followed by a solution of mercury(II) chloride (300 mg, 1.10 mmol) in a mixture of acetone/water (10:1, 2 mL) were added and the mixture was stirred at room temperature for 4 h. The mixture was concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate, 20:1 → 3:1) to give the title compound (540 mg, 96%) as a foam. IR (film): $\tilde{\nu}_{max}$ = 1716, 1450, 1262, 1109, 1090, 1069, 707 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.12–7.19 (m, 20 H, ArH), 5.87 (t, $J_{3,2}$ = $J_{3,4}$ = 3.9 Hz, 1 H, 3-Hβ), 5.74 (ddd, J = 1.0, 3.7 Hz, 1 H, 3-Hα), 5.54 (br. d, J = 3.7 Hz, 1 H, 1-Hα), 5.49 (dd, $J_{1,2}$ = 8.2, $J_{1,3}$ = 1.8 Hz, 1 H, 1-Hβ), 5.47–5.45 (m, 1 H, 4-Hα), 5.43–5.40 (m, 1 H, 4-Hβ), 5.34–5.32 (m, 1 H, 2-Hβ), 5.27–5.25 (m, 1 H, 2-Hα), 5.09–5.05 (m, 1 H, 5-Hα), 4.79 (dd, $J_{6,5}$ = 7.0, $J_{6,6'}$ = 11.3 Hz, 1 H, 6-Hβ), 4.73 (dd, $J_{6,5}$ = 7.1, $J_{6,6'}$ = 11.6 Hz, 1 H, 6-Hα), 4.71–4.68 (m, 1 H, 5-Hβ), 4.61 (dd, $J_{6',5}$ = 5.4, $J_{6,6'}$ = 11.3 Hz, 1 H, 6′-Hβ), 4.55 (dd, $J_{6',5}$ = 5.3, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-Hα) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.2 (C=O α), 166.2 (C=O β), 165.9 (C=O β), 165.4 (C=O α), 165.3 (C=O α), 165.2 (C=O β), 164.6 (C=O α), 164.1 (C=O β), 133.8, 133.6, 133.6, 133.5, 133.5, 133.4, 133.2, 133.2, 130.1–128.2 (aromatic protons), 92.6 (C-1α), 92.5 (C-1β), 72.3 (β), 68.8 (β), 68.3 (α),68.0 (β), 67.2 (α), 67.0 (α),66.6 (β), 64.9 (α),63.3 (C-6β), 63.1 (C-6α) ppm. $C_{34}H_{28}O_{10}$ (596.60): calcd. C 68.45, H 4.73; found C 68.40, H 4.77.

4.2.9. 2,3,4,6-Tetra-*O*-benzoyl-α,β-D-idopyranosyl Trichloroacetimidate (8): Obtained as described for the synthesis of **7**. D-Idopyranose **15** (540 mg, 0.91 mmol) was treated with trichloroacetonitrile (0.40 mL, 4.00 mmol) in the presence of potassium carbonate (100 mg, 0.72 mmol) and DBU (3 drops) in dichloromethane (20 mL) to give the title compound (533 mg, 80%) as a foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.80 (s, 1 H, NH-α), 8.61 (s, 1 H, NH-β), 8.22–7.16 (m, 20 H, ArH), 6.74 (d, $J_{1,2}$ = 3.0 Hz, 1 H, 1-Hβ), 6.64 (br. s, 1 H, 1-Hα), 6.23 (t, J = 7.7 Hz, 1 H, 3-Hβ), 5.73–5.71 (m, 1 H, 3-Hα), 5.64–5.68 (m, 2 H, 4-,2-Hβ), 5.47–5.45 (m, 1 H, 4-Hα), 5.45–5.43 (m, 1 H, 2-Hα), 5.08 (ddd, $J_{5,4}$ = 1.1, $J_{5,6}$ = 8.1, $J_{5,6'}$ = 4.4 Hz, 1 H, 5-Hα), 5.00–4.96 (m, 1 H, 5-Hβ), 4.87 (dd, $J_{6,5}$ = 6.6, $J_{6,6'}$ = 11.8 Hz, 1 H, 6-Hβ), 4.76 (dd, $J_{6',5}$ = 6.4, $J_{6,6'}$ = 11.8 Hz, 1 H, 6′-Hβ), 4.67 (dd, $J_{6,5}$ = 8.1, $J_{6,6'}$ = 11.7 Hz, 1 H, 6-Hα), 4.58 (dd, $J_{6',5}$ = 4.4, $J_{6,6'}$ = 11.7 Hz, 1 H, 6′-Hα) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.0 (α-C=O), 165.9 (β-C=O), 165.4 (β-C=O), 165.3 (β-C=O), 165.1 (α-C=O), 165.0 (β-C=O), 164.8 (α-C=O), 164.4 (α-C=O), 160.7 (β-C=N), 160.1 (α-C=N), 133.8, 133.7, 133.2, 130.4–128.2 (aromatic protons), 94.5 (C-1β), 94.2 (C-1α), 90.8 (C-Cl), 72.4 (β), 68.9 (β), 68.7 (β), 67.0 (β), 66.8 (α), 66.3 (α), 66.1 (α), 65.9 (α), 63.9 (C-6β), 63.3 (C-6α) ppm. $C_{36}H_{28}Cl_3NO_{10}$ (740.99): calcd. C 58.35, H 3.81, N 1.89, Cl 14.35; found C 58.13, H 3.88, N 1.87, Cl 14.43.

4.2.10. Synthesis of Saponins (Glycosylation); General Procedure: A solution of D-idopyranosyl trichloroacetimidate (**7** or **8**, 0.28 mmol) and a suitable triterpene acceptor (0.25 mmol) in dichloromethane (15 mL) was stirred for 20–30 min at room temperature over molecular sieves (4 Å, 300 mg, finely ground), then cooled to –40 °C and TMSOTf (30 μL, 0.15 mmol) was added. The mixture was stirred for 30 min, and the reaction was quenched with Et_3N (0.5 mL). The mixture was concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate, 10:1 → 7:3) to give the protected saponin as a foam.

4.2.10.1. 1-*O*-[3-β-Acetoxylup-20(29)-ene-28-oyl]-2,3,4,6-tetra-*O*-acetyl-α-D-idopyranosyl (20): Yield 89% starting from 3-*O*-acetylbetulinic acid (**1**) and 2,3,4,6-tetra-*O*-acetyl-D-idopyranosyl trichloroacetimidate (**7**). $[\alpha]_D^{20}$ = 33.7 (*c* = 0.3, chloroform). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.11 (br. d, $J_{1,2}$ = 1.3 Hz, 1 H, 1′-H), 5.11–5.08 (m, 1 H, 3′-H), 4.95–4.93 (m, 1 H, 4′-H), 4.85–4.83 (m, 1 H, 2′-H), 4.73 (br. s, 1 H, 29-H), 4.61 (br. s, 1 H, 29-H), 4.47 (dd, J = 6.1, 10.2 Hz, 1 H, 3-H), 4.42 (ddd, J = 2.3, 6.4 Hz, 1 H, 5′-H), 4.20–4.17 (m, 2 H, 6′-H, 6′-H), 3.00–2.93 (m, 1 H, 19-H), 2.33–2.21 (m, 3 H), 2.13 (s, 3 H, CH_3), 2.11 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3), 2.06 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3), 1.92–1.85 (m, 2 H), 1.68 (s, 3 H, CH_3), 0.97 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.84 (s, 6 H, 2× CH_3), 0.83 (s, 3 H, CH_3), 1.63–1.20 (m, 19 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2 (C=O), 171.0 (C=O), 170.3 (C=O), 169.6 (C=O), 169.0 (C=O), 168.7 (C=O), 150.0 (C-20), 109.9 (C-29), 89.8 ($^1J_{C1,H1}$ = 174 Hz, C-1′), 80.9 (C-3), 66.6, 66.4, 66.3, 66.0, 61.8 (C-6′), 56.7 (C), 55.5, 50.5, 49.2, 46.6, 42.4 (C), 40.7 (C), 38.4 (CH_2), 38.0, 37.8 (C), 37.1 (C), 36.6 (CH_2), 34.3 (CH_2), 32.0 (CH_2), 30.3 (CH_2), 29.5 (CH_2), 27.9, 25.4 (CH_2), 23.7 (CH_2), 21.3, 20.9 (CH_2), 20.7, 20.6, 20.6, 19.3, 18.1 (CH_2), 16.5, 16.2, 16.0, 14.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{46}H_{68}NaO_{13}$ $[M + Na]^+$ 851.4552; found 851.4545.

4.2.10.2. 1-*O*-[3-β-Acetoxylup-20(29)-ene-28-oyl]-2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl (21): Yield 86% starting from 3-*O*-acetylbetulinic acid (**1**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 34.1 (*c* = 0.25, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2948, 2871, 1728, 1451, 1263, 1250, 1107, 1069, 1027, 711 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.12–7.19 (m, 20 H, ArH), 6.48 (br. s, 1 H, 1′-H), 5.71–5.69 (m, 1 H, 3′-H), 5.52–5.50 (m, 1 H, 4′-H), 5.28–5.26 (m, 1 H, 2′-H), 4.90 (ddd, J = 1.9, 6.6 Hz, 1 H, 5′-H), 4.71 (br. s, 1 H, 29-H), 4.68 (dd, $J_{6,5}$ = 6.6, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.58 (br. s, 1 H, 29-H), 4.55 (dd, $J_{6',5}$ = 6.6, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.46 (dd, J = 5.8, 10.3 Hz, 1 H, 3-H), 2.98–2.92 (m, 1 H, 19-H), 2.34–2.29 (m, 2 H), 2.04 (s, 3 H, CH_3), 1.84–1.76 (m, 2 H), 1.64 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.83 (s, 3 H, CH_3), 0.82 (s, 3 H, CH_3), 0.78 (s, 3 H, CH_3), 1.62–1.10 (m, 20 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2 (C=O), 171.0 (C=O), 166.0 (C=O), 165.2 (C=O), 164.9 (C=O), 164.5 (C=O), 150.1 (C-20), 133.8, 133.6, 133.5, 133.2, 109.8 (C-29), 89.9 (C-1′), 80.9 (C-3), 66.8, 66.7, 66.5, 66.2, 62.5 (C-6′), 56.7 (C), 55.4, 50.5, 49.4, 46.4, 42.4 (C), 40.6 (C), 38.4 (CH_2), 37.8, 37.8 (C), 37.1 (C), 36.4 (CH_2), 34.1 (CH_2), 32.1 (CH_2), 30.3 (CH_2), 29.4 (CH_2), 27.9, 25.4 (CH_2), 23.7 (CH_2), 21.3, 20.8 (CH_2), 19.3, 18.2 (CH_2), 16.5, 16.2, 15.9, 14.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{66}H_{76}NaO_{13}$ $[M + Na]^+$ 1099.5184; found 1099.5179.

4.2.10.3. 3β-*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (23): Yield 97% starting from lupeol (**5**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 58.9 (*c* = 0.3, chloroform). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.18–

7.15 (m, 20 H, ArH), 5.59–5.58 (m, 1 H, 3′-H), 5.40 (br. s, 1 H, 4′-H), 5.32 (br. s, 1 H, 2′-H), 5.20–5.19 (m, 1 H, 1′-H), 5.00–4.96 (m, 1 H, 5′-H), 4.69 (d, J = 1.9 Hz, 1 H, 29-H), 4.66 (dd, $J_{6',5}$ = 8.0, $J_{6,6'}$ = 11.8 Hz, 1 H, 6′-H), 4.59 (dd, $J_{6',5}$ = 4.2, $J_{6,6'}$ = 11.8 Hz, 1 H, 6′-H), 4.57 (br. s, 1 H, 29-H), 3.35 (dd, J = 4.2, 11.8 Hz, 1 H, 3-H), 2.41–2.35 (m, 1 H), 1.96–1.89 (m, 1 H), 1.86–1.81 (m, 1 H), 1.69 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 0.79 (s, 6 H, 2× CH_3), 0.69 (s, 3 H, CH_3), 0.57–0.54 (m, 1 H), 1.68–0.75 (m, 21 H, lupane protons) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.2 (C=O), 164.7 (C=O), 150.9 (C-20), 133.5, 133.5, 133.2, 133.2, 109.4 (C-29), 93.8 ($^1J_{C1,H1}$ = 170 Hz, C-1′), 83.0 (C-3), 68.2, 67.2, 66.8, 64.6, 63.7 (CH_2), 55.8, 50.4, 48.3, 48.0, 43.0 (C), 42.8 (C), 40.8 (C), 40.0 (CH_2), 38.4 (C), 38.3 (CH_2), 38.0, 37.0 (C), 35.6 (CH_2), 34.2 (CH_2), 29.8 (CH_2), 28.6, 27.4 (CH_2), 25.1 (CH_2), 21.5 (CH_2), 20.9 (CH_2), 19.3, 18.2 (CH_2),18.0, 16.2, 16.1, 15.9, 14.7 ppm. HRMS (ESI): m/z calcd. for $C_{64}H_{76}NaO_{10}$ [M + Na]$^+$ 1027.5336; found 1027.5341. $C_{64}H_{76}O_{10}$·3/2H_2O (591.88): calcd. C 74.46, H 7.71; found C 74.63, H 7.73.

4.2.10.4. 3β-*O*-Acetyl-28-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (25): Yield 87% starting from 3-*O*-acetylbetulin (**2**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 45.9 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2947, 2871, 1727, 1451, 1264, 1249, 1108, 1028, 710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16–7.17 (m, 20 H, ArH), 5.63–5.62 (m, 1 H, 3′-H), 5.40 (br. s, 1 H, 4′-H), 5.27–5.25 (m, 1 H, 2′-H), 5.01 (s, 1 H, 1′-H), 4.87–4.83 (m, 1 H, 5′-H), 4.64–4.68 (br. s, 1 H, 29-H), 4.67–4.60 (m, 1 H, 6′-H), 4.62 (dd, $J_{6',5}$ = 6.3, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.58–4.57 (m, 1 H, 29-H), 4.45 (dd, J = 4.3, 11.0 Hz, 1 H, 3-H), 4.08–4.05 (m, 1 H, 28-H), 3.25 (d, J = 9.8 Hz, 1 H, 28-H), 2.39–2.30 (m, 1 H), 2.04 (s, 3 H, CH_3), 1.67 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.87 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.82 (s, 3 H, CH_3), 0.74 (s, 3 H, CH_3), 1.90–1.15 (m, 24 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (C=O), 166.0 (C=O), 165.3 (C=O), 165.1 (C=O), 164.5 (C=O), 150.3 (C-20), 133.5, 133.3, 133.2, 109.8 (C-29), 98.6 (C-1′), 80.9 (C-3), 67.4, 66.8, 66.5, 64.4, 62.9 (C-6), 55.3, 50.2, 48.8, 48.1, 47.0 (C), 42.6 (C), 40.8 (C), 38.3 (CH_2), 37.8 (C), 37.5, 37.0 (C), 34.5 (CH_2), 34.0 (CH_2), 29.8 (CH_2), 27.9 (CH, CH_2), 27.2 (CH_2), 25.0 (CH_2), 23.6 (CH_2), 21.3, 20.6 (CH_2), 19.0, 18.1 (CH_2), 16.4, 16.1, 15.9, 14.7 ppm. HRMS (ESI): m/z calcd. for $C_{66}H_{78}NaO_{12}$ [M + Na]$^+$ 1085.5391; found 1085.5386. $C_{66}H_{78}O_{12}$·0.5H_2O (1072.36): calcd. C 73.92, H 7.43; found C 73.76, H 7.34.

4.2.10.5. 28-*O*-Acetyl-3β-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (27): Yield 81% starting from 28-*O*-acetylbetulin (**3**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 54.9 (c = 0.2, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2945, 2871, 1726, 1452, 1265, 1248, 1107, 1028, 711 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16–7.15 (m, 20 H, ArH), 5.59–5.57 (m, 1 H, 3′-H), 5.41 (br. s, 1 H, 4′-H), 5.32 (s, 1 H, 1′-H), 5.20–5.18 (m, 1 H, 2′-H), 4.98 (ddd, J = 1.5, 4.2, 7.2 Hz, 1 H, 5′-H), 4.69–4.67 (m, 1 H, 29-H), 4.67 (dd, $J_{6,5}$ = 8.2, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.56–4.60 (m, 2 H, 6′-H, 29-H), 4.27–4.24 (m, 1 H, 28-H), 3.85 (d, J = 11.1 Hz, 1 H, 28-H), 3.34 (dd, J = 4.4, 11.8 Hz, 1 H, 3-H), 2.48–2.41 (m, 1 H), 2.07 (s, 3 H, CH_3), 2.00–1.92 (m, 1 H), 1.70 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 1.01 (s, 3 H, CH_3), 0.98 (s, 3 H, CH_3), 0.78 (s, 3 H, CH_3), 0.69 (s, 3 H, CH_3), 1.85–0.55 (m, 23 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6 (C=O), 166.1 (C=O), 165.3 (C=O), 165.2 (C=O), 164.7 (C=O), 150.1 (C-20), 109.9 (C-29), 93.9 (C-1′), 83.1 (C-3), 68.2, 67.2, 66.8, 64.6, 63.7 (CH_2), 62.8 (CH_2), 55.8, 50.3, 48.8, 47.7, 46.3 (C), 42.7 (C), 40.9 (C), 38.4 (CH_2), 38.3 (C), 37.5, 37.0 (C), 34.6 (CH_2), 34.2 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 28.6, 27.1 (CH_2), 25.2 (CH_2), 21.6 (CH_2), 21.0, 20.8 (CH_2), 19.2, 18.2 (CH_2), 16.2, 16.1, 16.0, 14.9 ppm. HRMS (ESI): m/z calcd. for $C_{66}H_{78}NaO_{12}$ [M + Na]$^+$ 1085.5391; found 1085.5397. $C_{66}H_{78}O_{12}$·H_2O (1081.37): calcd. C 73.31, H 7.46; found C 73.22, H 7.66.

4.2.10.6. 28-*O*-*tert*-Butyldiphenylsilyl-3β-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (29): Yield 87% starting from 28-*O*-*tert*-butyldiphenylsilylbetulin (**4**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 35.4 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2942, 2860, 1727, 1452, 1265, 1108, 1027, 709 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16–7.12 (m, 30 H, ArH), 5.59–5.57 (m, 1 H, 3′-H), 5.40 (br. s, 1 H, 4′-H), 5.31 (s, 1 H, 1′-H), 5.19–5.18 (m, 1 H, 2′-H), 4.99–4.95 (m, 1 H, 5′-H), 4.66 (dd, $J_{6,5}$ = 8.2, $J_{6,6'}$ = 11.6 Hz, 1 H, 6′-H), 4.56–4.60 (m, 2 H, 6′-H, 29-H), 4.53 (br. s, 1 H, 29-H), 3.70–3.66 (m, 1 H, 28-H), 3.31–3.35 (m, 2 H, 3-H, 28-H), 2.29–2.23 (m, 1 H), 2.16–2.10 (m, 1 H), 1.87–1.78 (m, 2 H), 1.54 (s, 3 H, CH_3), 1.06 (s, 9 H, *tert*-butyl), 1.01 (s, 3 H, CH_3), 0.93 (s, 3 H, CH_3), 0.72 (s, 3 H, CH_3), 0.68 (s, 3 H, CH_3), 0.67 (s, 3 H, CH_3), 1.53–0.78 (m, 18 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.2 (C=O), 164.7 (C=O), 150.8 (C-20), 135.6, 133.8, 133.5, 133.2, 130.2, 109.4 (C-29), 93.8 (C-1′), 83.0 (C-3), 77.2, 68.2, 67.3, 66.8, 64.6, 63.7 (CH_2), 61.1 (CH_2), 55.8, 50.3, 48.5, 48.4 (C), 47.8, 42.6 (C), 40.8 (C), 38.4 (C), 38.2 (CH_2), 37.2, 37.0 (C), 34.5 (CH_2), 34.1 (CH_2), 29.9 (CH_2), 29.5 (CH_2), 28.6, 27.1 (CH_2), 26.9 (3× CH_3), 25.1 (CH_2), 21.5 (CH_2), 20.7 (CH_2), 19.4 (C), 19.1, 18.2 (CH_2), 16.2, 16.0, 15.7, 14.9 ppm. $C_{80}H_{91}O_{11}Si$ (1259.72): calcd. C 76.28, H 7.52; found C 76.41, H 7.40.

4.2.10.7. 3β-*O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-en-28-ol (30): To a solution of **29** (440 mg, 0.35 mmol) in THF (12 mL), tetrabutylammonium fluoride (1 M in THF, 1.75 mL, 1.75 mmol) was added followed by acetic acid (100 μL, 1.75 mmol) and the mixture was stirred in a sealed tube at 60 °C for 17 h. The mixture was then concentrated and the residue was purified by column chromatography (hexane/ethyl acetate, 7:1→3:1) to give the title compound (279 mg, 78%) as a foam. $[\alpha]_D^{20}$ = 48.9 (c = 0.4, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2942, 2871, 1726, 1451, 1265, 1107, 1027, 710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.16–7.14 (m, 20 H, ArH), 5.59–5.57 (m, 1 H, 3′-H), 5.41 (br. s, 1 H, 4′-H), 5.32 (s, 1 H, 1′-H), 5.20–5.18 (m, 1 H, 2′-H), 5.00–4.96 (m, 1 H, 5′-H), 4.65–4.70 (m, 2 H, 6′-H, 29-H), 4.56–4.60 (m, 2 H, 6′-H, 29-H), 3.79 (d, J = 10.2 Hz, 1 H, 28-H), 3.32–3.36 (m, 2 H, 3-H, 28-H), 2.42–2.35 (m, 1 H), 1.96–1.82 (m, 2 H), 1.70 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 0.98 (s, 3 H, CH_3), 0.78 (s, 3 H, CH_3), 0.69 (s, 3 H, CH_3), 1.72–0.95 (m, 23 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 165.3 (C=O), 165.2 (C=O), 164.7 (C=O), 150.4 (C-20), 133.5, 133.2, 109.7 (C-29), 93.8 (C-1′), 83.0 (C-3), 77.2, 68.2, 67.2, 66.8, 64.6, 63.7 (CH_2), 60.5 (CH_2), 55.8, 50.3, 48.8, 47.8 (C, CH), 42.7 (C), 40.9 (C), 38.4 (C), 38.3 (CH_2), 37.3, 37.0 (C), 34.2 (CH_2), 34.0 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 28.6, 27.0 (CH_2), 25.2 (CH_2), 21.5 (CH_2), 20.8 (CH_2), 19.1, 18.2 (CH_2), 16.2, 16.1, 15.9, 14.9 ppm. HRMS (ESI): m/z calcd. for $C_{64}H_{76}NaO_{11}$ [M + Na]$^+$ 1043.5285; found 1043.5289.

4.2.10.8. 3β,28-Di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (32): Yield 97% starting from **30** and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 51.0 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2946, 2871, 1726, 1452, 1266, 1108, 1028, 757, 710 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ [sugar protons with prime (′) mark belong to idose ring at position 3 of lupane, with double prime (″) to idose ring at position 28 of lupane] = 8.16–6.95 (m, 40 H, ArH), 5.64–5.62 (m, 1 H, 3″-H), 5.60–5.58 (m, 1 H, 3′-H), 5.41 (br. s, 2 H, 4′,4″-H), 5.31 (s, 1 H, 1′-H),

5.27–5.26 (m, 1 H, 2′′-H), 5.20–5.19 (m, 1 H, 2′-H), 5.10 (s, 1 H, 1′′-H), 5.00–4.96 (m, 1 H, 5′-H), 4.87–4.84 (m, 1 H, 5′′-H), 4.56–4.69 (m, 5 H, 6′,6′′,6′′′,6′′′′,29-H), 4.05 (br. d, J = 9.3 Hz, 1 H, 28-H), 3.34 (dd, J = 4.3, 11.7 Hz, 1 H, 3-H), 3.26 (d, J = 9.7 Hz, 1 H, 28-H), 2.38–2.33 (m, 1 H), 1.68 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 0.68 (s, 3 H, CH_3), 0.67 (s, 3 H, CH_3), 2.00–0.85 (m, 25 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.1 (C=O), 166.0 (C=O), 165.3 (C=O), 165.3 (C=O), 165.2 (C=O), 165.2 (C=O), 164.7 (C=O), 164.5 (C=O), 150.2 (lupane C-20), 133.5, 133.3, 133.2, 109.8 (lupane C-29), 98.7 (C-1), 93.9 (C-1), 83.1 (lupane C-3), 77.2, 68.2, 67.4, 67.2, 67.2 (CH_2), 66.9, 66.8, 66.5, 64.6, 64.5, 63.6 (CH_2), 62.9 (CH_2), 55.8, 50.3, 48.8, 48.0, 47.1 (C), 42.7 (C), 40.8 (C), 38.4 (C), 38.2 (CH_2), 37.5, 37.0 (C), 34.5 (CH_2), 34.1 (CH_2), 29.8 (CH_2), 28.6, 27.2 (CH_2), 25.0 (CH_2), 21.5 (CH_2), 21.3, 20.6 (CH_2), 19.0, 18.2 (CH_2), 16.2, 16.0, 15.9, 14.9 ppm. HRMS (ESI): m/z calcd. for $C_{98}H_{102}NaO_{20}$ $[M + Na]^+$ 1621.6862; found 1621.6917.

4.2.10.9. 28-*O*-Acetyl-3α-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-idopyranosyl)lup-20(29)-ene (34): Yield 52% starting from 28-*O*-acetyl-*epi*-betulin (**6**) and 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate (**8**). $[\alpha]_D^{20}$ = 23.0 (c = 0.25, chloroform). IR (film): $\tilde{\nu}_{max}$ = 2945, 2871, 1727, 1451, 1265, 1247, 1107, 1028, 710 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.15–7.12 (m, 20 H, ArH), 5.62–5.60 (m, 1 H, 3′-H), 5.39 (br. s, 1 H, 4′-H), 5.35 (br. s, 1 H, 2′-H), 5.20 (s, 1 H, 1′-H), 5.04–5.00 (m, 1 H, 5′-H), 4.74 (br. d, J = 3.5 Hz, 1 H, 29-H), 4.67–4.72 (m, 2 H, 6′,29-H), 4.59 (dd, $J_{6',5}$ = 4.8, $J_{6,6'}$ = 11.7 Hz, 1 H, 6′-H), 4.21 (d, J = 11.0 Hz, 1 H, 28-H), 3.85 (d, J = 11.0 Hz, 1 H, 28-H), 3.44 (br. s, 1 H, 3-H), 2.47–2.41 (m, 1 H), 2.06 (s, 3 H, CH_3), 2.02–1.93 (m, 1 H), 1.77 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3), 0.90 (s, 3 H, CH_3), 0.78 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 0.67 (s, 3 H, CH_3), 1.85–0.85 (m, 23 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6 (C=O), 166.2 (C=O), 165.4 (C=O), 165.1 (C=O), 164.6 (C=O), 150.3 (C-20), 133.6, 133.5, 109.8 (C-29), 99.7 (C-1′), 85.0 (C-3), 67.6, 67.5, 67.4, 65.0, 63.5 (CH_2), 62.8 (CH_2), 49.8, 49.6, 48.9, 47.7, 46.3 (C), 42.6 (C), 40.9 (C), 38.3 (C), 37.4 (CH_2), 37.1 (C), 34.5 (CH_2), 34.1 (CH_2), 33.7 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 28.1, 27.0 (CH_2), 25.2 (CH_2), 24.4 (CH_2), 22.2, 21.0, 20.4 (CH_2), 19.4, 17.8 (CH_2), 16.0, 15.9, 14.7 ppm. HRMS (ESI): m/z calcd. for $C_{66}H_{78}NaO_{12}$ $[M + Na]^+$ 1085.5391; found 1085.5419. $C_{66}H_{78}O_{12}\cdot H_2O$ (1081.37): calcd. C 73.31, H 7.46; found C 73.54, H 7.48.

4.2.11. Debenzoylation of Glycosides; General Procedure (Method A): A suspension of protected saponin (0.20 mM) and K_2CO_3 (50 mg) in MeOH (10 mL) was stirred for 1 h, then neutralized with Amberlyst 15 resin (H^+ form), filtered through a PTFE syringe filter, and concentrated. The residue was purified by column chromatography (hexane/ethyl acetate, 3:1→ethyl acetate) to give unprotected saponin as an amorphous powder.

4.2.11.1. 1-*O*-[3-β-Acetoxylup-20(29)-ene-28-oyl]-α-D-idopyranosyl (22): Yield 69% starting from **21**. This compound was also obtained from acetate **20** by treatment with sodium methoxide (Method B): To a suspension of **20** (166 mg, 0.20 mM) in MeOH (10 mL), sodium methoxide (0.95 M in methanol, 50 μL) was added and the mixture was stirred at room temperature for 2 h. The mixture was neutralized with Amberlyst 15 resin (H^+ form), filtered through a PTFE syringe filter, concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate, 3:1→ethyl acetate) to give the title compound (110 mg, 83%) as an amorphous powder. $[\alpha]_D^{20}$ = 43.9 (chloroform). IR (film): $\tilde{\nu}_{max}$ = 3418 (br), 2947, 2872, 1726, 1376, 1072, 1031, 978, 757 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.19 (br. s, 1 H, 1′-H), 4.73 (br. s, 1 H, 29-H), 4.61 (br. s, 1 H, 29-H), 4.46 (dd, J = 5.9, 10.4 Hz, 1 H, 3-H), 3.98–4.13 (m, 5 H), 3.64 (br. s, 1 H), 3.04–2.93 (m, 1 H), 2.34–2.18 (m, 3 H), 2.05 (s, 3 H, CH_3), 1.68 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.83 (s, 3 H, CH_3), 1.93–1.18 (m, 25 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.9 (C=O), 171.1 (C=O), 150.0 (C-20), 109.9 (C-29), 93.9 (C-1′), 80.9 (C-3), 72.0, 68.4, 68.3, 67.0, 64.9 (C-6′), 56.9 (C), 55.4, 50.5, 49.3, 46.6, 42.5 (C), 40.7 (C), 38.4 (CH_2), 38.1, 37.8 (C), 37.1 (C), 36.8 (CH_2), 34.3 (CH_2), 32.3 (CH_2), 30.3 (CH_2), 29.6 (CH_2), 27.9, 25.4 (CH_2), 23.7 (CH_2), 21.3, 20.9 (CH_2), 19.3, 18.2 (CH_2), 16.5, 16.2, 14.7 ppm. HRMS (ESI): m/z calcd. for $C_{38}H_{60}NaO_9$ $[M + Na]^+$ 683.4135; found 683.4139. $C_{38}H_{60}O_9\cdot 1.5H_2O$ (687.92): calcd. C 66.35, H 9.23; found C 66.71, H 9.41.

4.2.11.2. 3β-*O*-(α-D-Idopyranosyl)lup-20(29)-ene (24): Yield 81% starting from **23**. $[\alpha]_D^{20}$ = 73.4 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3402 (br), 2943, 2871, 1055, 1016, 882, 759 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.13 (s, 1 H), 4.69 (br. d, J = 2.2 Hz, 1 H), 4.58–4.56 (m, 1 H), 4.16 (br. s, 1 H), 4.07–3.99 (m, 3 H), 3.95–3.90 (m, 1 H), 3.65 (br. s, 1 H), 3.53 (d, J = 9.1 Hz, 1 H, 29-H), 3.30 (dd, J = 4.2, 11.8 Hz, 1 H, 3-H), 2.41–2.34 (m, 1 H), 1.97–1.88 (m, 1 H), 1.68 (s, 3 H, CH_3), 1.03 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.79 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 0.74–0.70 (m, 1 H), 1.80–0.75 (m, 25 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 150.9 (C-20), 109.4 (C-29), 98.4 (C-1′), 84.9 (C-3), 72.7, 68.5, 68.2, 65.3 (CH_2), 65.1, 55.5, 50.4, 48.3, 48.0, 43.0 (C), 42.8 (C), 40.8 (C), 40.0 (CH_2), 38.3 (C), 38.2 (CH_2), 38.0, 37.1 (C), 35.6 (CH_2), 34.2 (CH_2), 29.8 (CH_2), 28.7, 27.4 (CH_2), 25.1 (CH_2), 22.3 (CH_2), 20.9 (CH_2), 19.3, 18.3 (CH_2),18.0, 16.5, 16.0, 16.0, 14.5 ppm. HRMS (ESI): m/z calcd. for $C_{36}H_{60}NaO_6$ $[M + Na]^+$ 611.4288; found 611.4296.

4.2.11.3. 3β-*O*-Acetyl-28-*O*-(α-D-idopyranosyl)lup-20(29)-ene (26): Yield 76% starting from **25**. $[\alpha]_D^{20}$ = 46.6 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3434 (br), 2945, 2872, 1727, 1455, 1375, 1250, 1090, 1060, 1028, 980, 757 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.94 (s, 1 H, 1′-H), 4.70 (br. d, J = 1.9 Hz, 1 H), 4.62–4.60 (m, 1 H), 4.48 (dd, J = 5.3, 10.9 Hz, 1 H, 3-H), 4.01–4.13 (m, 4 H), 4.00–3.98 (m, 1 H), 3.93 (br. s, 1 H), 3.73–3.71 (m, 1 H), 3.09 (d, J = 9.3 Hz, 1 H, 28-H), 2.42–2.36 (m, 1 H), 2.04 (s, 3 H, CH_3), 1.98–1.83 (m, 2 H), 1.68 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 0.97 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 1.75–1.15 (m, 26 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.1 (C=O), 149.9 (C-20), 110.0 (C-29), 102.4 (C-1′), 80.9 (C-3), 72.5, 68.2, 67.7, 67.3 (CH_2), 65.4 (CH_2), 64.8, 55.3, 50.2, 48.8, 47.8, 46.7 (C), 42.7 (C), 40.9 (C), 38.3 (CH_2), 37.8 (C), 37.6, 37.1 (C), 34.4 (CH_2), 34.2 (CH_2), 30.2 (CH_2), 29.6 (CH_2), 27.9, 27.1 (CH_2), 25.1 (CH_2), 23.6 (CH_2), 21.3, 20.8 (CH_2), 19.1, 18.1 (CH_2), 16.5, 16.1, 16.1, 14.8 ppm. HRMS (ESI): m/z calcd. for $C_{38}H_{62}NaO_8$ $[M + Na]^+$ 669.4342; found 669.4344.

4.2.11.4. 28-*O*-Acetyl-3β-*O*-(α-D-idopyranosyl)lup-20(29)-ene (28): Yield 82% starting from **27**. $[\alpha]_D^{20}$ = 54.1 (c = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3407 (br), 2943, 2871, 1738, 1455, 1389, 1364, 1240, 1030, 757 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.13 (br. s, 1 H), 4.70–4.68 (m, 1 H), 4.59 (br. s, 1 H), 4.16–4.26 (m, 3 H), 3.99–4.07 (m, 3 H), 3.95–3.92 (m, 1 H), 3.85 (d, J = 11.2 Hz, 1 H, 28-H), 3.65 (br. s, 1 H), 3.52–3.49 (m, 1 H), 3.29 (dd, J = 4.2, 11.7 Hz, 1 H, 3-H), 2.47–2.41 (m, 1 H), 2.07 (s, 3 H, CH_3), 2.01–1.91 (m, 1 H), 1.68 (s, 3 H, CH_3), 1.03 (s, 3 H, CH_3), 0.97 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 1.85–0.72 (m, 25 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.7 (C=O), 150.1 (C-20), 109.9 (C-29), 98.4 (C-1′), 84.9 (C-3), 72.6, 68.5, 68.3, 65.3 (CH_2), 65.1, 62.8 (CH_2), 55.5, 50.3, 48.8, 47.7, 46.3 (C), 42.7 (C), 40.9 (C), 38.3 (C), 38.2 (CH_2), 37.5, 37.0 (C),

34.5 (CH_2), 34.1 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 28.7, 27.0 (CH_2), 25.1 (CH_2), 22.3 (CH_2), 21.0, 20.8 (CH_2), 19.1, 18.2 (CH_2), 16.5, 16.0, 16.0, 14.7 ppm. HRMS (ESI): *m/z* calcd. for $C_{38}H_{62}NaO_8$ [M + Na]$^+$ 669.4342; found 669.4346. $C_{38}H_{62}O_8{\cdot}2H_2O$ (682.95): calcd. C 66.83, H 9.74; found C 66.81, H 9.70.

4.2.11.5. 3β-*O*-(α-D-Idopyranosyl)lup-20(29)-en-28-ol (31): Yield 60% starting from **30**. $[\alpha]_D^{20}$ = 67.3 (*c* = 0.25, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3420 (br), 2940, 2869, 1672, 1641, 1454, 1018, 880 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$ + 10% CD_3OD): δ = 5.01 (br. s, 1 H), 4.68 (br. d, *J* = 1.7 Hz, 1 H), 4.58 (br. s, 1 H), 4.18–4.15 (m, 1 H), 3.89–3.93 (m, 3 H), 3.77 (d, *J* = 10.7 Hz, 1 H, 28-H), 3.63 (br. s, 1 H), 3.28–3.33 (m, 1 H), 1.84–2.00 (m, 3 H), 1.68 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 0.98 (s, 6 H, 2× CH_3), 0.83 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 1.75–0.75 (m, 29 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$ + 10% CD_3OD): δ = 150.4 (C-20), 109.6 (C-29), 97.9 (C-1′), 84.6 (C-3), 71.2, 68.3, 65.8, 63.8 (CH_2), 60.1 (CH_2), 55.4, 50.3, 48.7, 47.7, 47.6 (C), 42.6 (C), 40.8 (C), 38.2 (C), 38.1 (CH_2), 37.2, 37.0 (C), 34.1 (CH_2), 33.9 (CH_2), 29.6 (CH_2), 29.0 (CH_2), 28.6, 26.9 (CH_2), 25.1 (CH_2), 22.1 (CH_2), 20.8 (CH_2), 19.0, 18.2 (CH_2), 16.4, 15.9, 15.9, 14.6 ppm. HRMS (ESI): *m/z* calcd. for $C_{36}H_{60}NaO_7$ [M + Na]$^+$ 627.4237; found 627.4223.

4.2.11.6. 3β,28-Di-*O*-(α-D-idopyranosyl)lup-20(29)-ene (33): Yield 40% starting from **32**. $[\alpha]_D^{20}$ = 61.9 (*c* = 0.25, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3333 (br), 2938, 2871, 1725, 1642, 1456, 1056, 1018, 979, 880 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$ + 10% CD_3OD): δ = 5.01 (s, 1 H), 4.91 (s, 1 H), 4.69 (br. s, 1 H), 4.60 (br. s, 1 H), 4.18–4.15 (m, 1 H), 3.89–4.03 (m, 8 H), 3.72–3.70 (m, 1 H), 3.65–3.63 (m, 1 H), 3.31 (dd, *J* = 4.2, 11.6 Hz, 1 H, 3-H), 3.09 (d, *J* = 9.5 Hz, 1 H, 28-H), 1.68 (s, 3 H, CH_3), 1.03 (s, 3 H, CH_3), 0.98 (s, 6 H, 2× CH_3), 0.84 (s, 3 H, CH_3), 0.76 (s, 3 H, CH_3), 1.98–0.70 (m, 26 H, lupane protons) ppm. ^{13}C NMR (125 MHz, $CDCl_3$ + 10% CD_3OD): δ = 150.0 (lupane C-20), 109.9 (lupane C-29), 101.9 (C-1), 97.9 (C-1), 84.5 (lupane C-3), 71.2, 71.2, 68.4, 68.4, 68.3, 67.6, 67.1 (CH_2), 65.8, 65.5, 63.9 (CH_2), 63.8 (CH_2), 55.4, 50.2, 48.7, 47.7, 46.6 (C), 42.7 (C), 40.8 (C), 38.2 (C), 38.1 (CH_2), 37.4, 37.0 (C), 34.3 (CH_2), 34.1 (CH_2), 30.1 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 28.6, 26.9 (CH_2), 25.0 (CH_2), 22.1 (CH_2), 20.8 (CH_2), 19.0, 18.1 (CH_2), 16.4, 15.9, 14.7 ppm. HRMS (ESI): *m/z* calcd. for $C_{42}H_{70}NaO_{12}$ [M + Na]$^+$ 789.4765; found 789.4754. $C_{42}H_{70}O_{12}{\cdot}H_2O$ (785.04): calcd. C 64.26, H 9.24; found C 64.35, H 9.26.

4.2.11.7. 28-*O*-Acetyl-3α-*O*-(α-D-idopyranosyl)lup-20(29)-ene (35): Yield 81% starting from **34**. $[\alpha]_D^{20}$ = 16.2 (*c* = 0.3, chloroform). IR (film): $\tilde{\nu}_{max}$ = 3434 (br), 2942, 2871, 1454, 1242, 1086, 1059, 1028, 757 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.04 (br. s, 1 H), 4.68–4.67 (m, 1 H), 4.58 (br. s, 1 H), 4.24 (d, *J* = 11.0 Hz, 1 H, 28-H), 4.04–4.11 (m, 4 H), 3.96 (br. s, 1 H), 3.84 (d, *J* = 11 Hz, 1 H, 28-H), 3.80–3.79 (m, 1 H), 3.13 (br. s, 1 H, 3-H), 2.46–2.41 (m, 1 H), 2.07 (s, 3 H, CH_3), 1.75–2.00 (m, 4 H), 1.67 (s, 3 H, CH_3), 1.02 (s, 3 H, CH_3), 0.96 (s, 3 H, CH_3), 0.92 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 0.84 (s, 3 H, CH_3), 1.65–0.85 (m, 24 H, lupane protons) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.7 (C=O), 150.2 (C-20), 109.8 (C-29), 104.7 (C-1′), 88.1 (C-3), 72.8, 68.3, 67.6, 65.4 (CH_2), 65.0, 62.9 (CH_2), 50.6, 50.5, 48.8, 47.8 (C), 47.7, 46.3 (C), 42.7 (C), 41.0 (C), 38.2 (C), 37.5, 37.1 (C), 34.9 (CH_2), 34.5 (CH_2), 34.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 28.5, 27.0 (CH_2), 25.1 (CH_2), 24.2 (CH_2), 21.9, 21.1, 20.7 (CH_2), 19.1, 18.0 (CH_2), 16.0, 16.0, 14.9 ppm. HRMS (ESI): *m/z* calcd. for $C_{38}H_{62}NaO_8$ [M + Na]$^+$ 669.4342; found 669.4348. $C_{38}H_{62}O_8{\cdot}2H_2O$ (682.95): calcd. C 66.83, H 9.74; found C 66.38, H 9.75.

4.3. Biological Evaluation

4.3.1. Cell culture: Stock solutions (10 mmol/L) of tested compounds were prepared by dissolving an appropriate quantity of each substance in dimethyl sulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Invitrogen Corporation, CA, USA). The screening cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF7, cervical carcinoma cell line HeLa, and human fibroblasts BJ) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/L), penicillin (10000 U) and streptomycin (10 mg/mL). The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment. Cells were subcultured two or three times a week by using the standard trypsinization procedure.

4.3.2. Calcein AM assay: Suspensions of tested cell lines (ca. 1.0×10^5 cells/mL) were placed in 96-well microtiter plates and, after 24 h stabilization (time zero), the tested compounds were added (in three 20 μL aliquots) in serially diluted concentrations in DMSO. Control cultures were treated with DMSO alone, and the final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The test compounds were typically evaluated at six threefold dilutions and the highest final concentration was generally 50 μM. After 72 h incubation, Calcein AM solution (100 μL, Molecular Probes, Invitrogen, CA, USA) was added, and incubation was continued for 1 h. The fluorescence of viable cells was then quantified by using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from control wells and multiplying by 100. These ratios were then used to construct dose-response curves from which IC_{50} values, the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated. The results obtained for selected compounds are shown in Table 1.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra are provided.

Acknowledgments

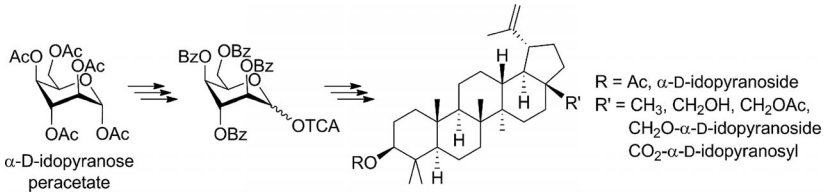
This work was financed by the National Science Centre, Poland (grant number 2012/07/B/ST5/00823), by the Czech Ministry of Education (National Program for Sustainability I, grant number LO1204) and by the Grant Agency of the Czech Republic (grant number 14-19590S). The authors thank Olga Hustáková for excellent technical assistance.

[1] S. B. Mahato, A. N. Ganguly, N. P. Sahu, *Phytochemistry* **1982**, *21*, 959.
[2] K. Hostettmann, A. Marston, in: *Saponins*, Cambridge University Press, Cambridge, UK, **1995**.
[3] C. Gauthier, J. Legault, M. Piochon-Gauthier, A. Pichette, *Phytochem. Rev.* **2011**, *10*, 521–544.
[4] R.-Y. Kuo, K. Qian, S. L. Morris-Natschke, K.-H. Lee, *Nat. Prod. Rep.* **2009**, *26*, 1321–1344.
[5] C. Bachran, S. Bachran, M. Sutherland, D. Bachran, H. Fuchs, *Mini-Rev. Med. Chem.* **2008**, *8*, 575–584.
[6] D. Yu, S. L. Morris-Natschke, K.-H. Lee, *Med. Res. Rev.* **2007**, *27*, 108–132.
[7] H. Sun, W.-S. Fang, *Bot. Stud.* **2006**, *47*, 339–368.
[8] S. G. Sparg, M. E. Light, J. van Staden, *J. Ethnopharmacol.* **2004**, *94*, 219–243.

[9] A. V. Rao, D. M. Gurfinkel, *Drug Metab. Drug Interact.* **2000**, *17*, 211–235.
[10] K. Oda, H. Matsuda, T. Murakami, S. Katayama, T. Ohgitani, M. Yoshikawa, *Biol. Chem.* **2000**, *381*, 67–74.
[11] S. Man, W. Gao, Y. Zhang, L. Huang, C. Liu, *Fitoterapia* **2010**, *81*, 703–714.
[12] Y. Wang, Y. Zhang, Z. Zhu, S. Zhu, Y. Li, M. Li, B. Yu, *Bioorg. Med. Chem.* **2007**, *15*, 2528–2532.
[13] D. Thibeault, C. Gauthier, J. Legault, J. Bouchard, P. Dufour, A. Pichette, *Bioorg. Med. Chem.* **2007**, *15*, 6144–6157.
[14] J. Gao, X. Li, G. Gu, B. Sun, M. Cui, M. Ji, H.-X. Lou, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 622–627.
[15] C. Gauthier, J. Legault, M. Piochon, S. Lavoie, S. Tremblay, A. Pichette, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2310–2314.
[16] C. Gauthier, J. Legault, A. Pichette, *Mini-Rev. Org. Chem.* **2009**, *6*, 321–344.
[17] C. Gauthier, J. Legault, S. Rondeau, A. Pichette, *Tetrahedron Lett.* **2009**, *50*, 988–991.
[18] O. B. Flekhter, L. A. Baltina, G. A. Tolstikov, *J. Nat. Prod.* **2000**, *63*, 992–994.
[19] L. E. Obinokova, M. W. Denisenko, W. A. Denisenko, N. I. Uvarova, *Khim. Prir. Soedin.* **1988**, 212–217.
[20] L. E. Obinokova, G. I. Oshitok, W. A. Denisenko, W. F. Anufriev, A. M. Tolkatz, N. I. Uvarowa, *Khim. Prir. Soedin.* **1984**, 182–187.
[21] N. I. Uvarova, L. N. Atopkina, G. B. Elyakov, *Carbohydr. Res.* **1980**, *83*, 33–42.
[22] N. I. Uvarova, G. I. Oshitok, G. B. Elyakov, *Carbohydr. Res.* **1973**, *27*, 79–87.
[23] N. I. Uvarova, G. I. Oshitok, A. K. Dzizenko, V. V. Isakov, G. B. Elyakov, *Dokl. Akad. Nauk SSSR* **1972**, *202*, 368–370.
[24] Z. Pakulski, *Pol. J. Chem.* **2005**, *79*, 361–367.
[25] P. Cmoch, Z. Pakulski, J. Swaczynová, M. Strnad, *Carbohydr. Res.* **2008**, *343*, 995–1003.
[26] P. Cmoch, A. Korda, L. Rárová, J. Oklešťková, M. Strnad, R. Luboradzki, Z. Pakulski, *Tetrahedron* **2014**, *70*, 2717–2730.
[27] I. Capila, R. J. Linhardt, *Angew. Chem. Int. Ed.* **2002**, *41*, 390–412; *Angew. Chem.* **2002**, *114*, 426.
[28] R. Lever, C. P. Page, *Nature Rev. Drug Discov.* **2002**, *1*, 140–148.
[29] Y.-H. Chen, F. Poly, Z. Pakulski, P. Guerry, M. A. Monteiro, *Carbohydr. Res.* **2008**, *343*, 1034–1040.
[30] G. Bianchi, A. Gamba, C. Murelli, F. Salamini, D. Bartels, *Plant J.* **1991**, *1*, 355–359.
[31] O. W. Howarth, N. Pozzi, G. Vlahov, D. Bartels, *Carbohydr. Res.* **1996**, *289*, 137–142.
[32] P. Guerry, F. Poly, M. Riddle, A. C. Maue, Y.-H. Chen, M. A. Monteiro, *Front. Cell. Infect. Microbiol.* **2012**, *2*, Article 7, DOI: 10.3389/fcimb.2012.00007.
[33] H. Paulsen, W.-P. Trautwein, F. Garrido Espinosa, K. Heyns, *Chem. Ber.* **1967**, *100*, 2822–2836.
[34] H. Paulsen, *Methods Carbohydr. Chem.* **1972**, *6*, 142–149.
[35] T. Chiba, S. Tejima, *Chem. Pharm. Bull.* **1977**, *25*, 1049–1054.
[36] T. Chiba, S. Tejima, *Chem. Pharm. Bull.* **1978**, *26*, 3426–3432.
[37] M. Dromowicz, P. Köll, *Carbohydr. Res.* **1998**, *308*, 169–171.
[38] R. Hevey, A. Morland, C.-C. Ling, *J. Org. Chem.* **2012**, *77*, 6760–6772.
[39] S. Kopitzki, J. Thiem, *Eur. J. Org. Chem.* **2013**, 4008–4016.
[40] R. Blattner, R. H. Furneaux, Z. Pakulski, *Carbohydr. Res.* **2006**, *341*, 2115–2125.
[41] R. Bukowski, L. M. Morris, R. I. Woods, T. Weimar, *Eur. J. Org. Chem.* **2001**, 2697–2705.
[42] I. A. Ivanova, A. J. Ross, M. A. J. Ferguson, A. V. Nikolaev, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1743–1753.
[43] H. Paulsen, C.-P. Herold, *Chem. Ber.* **1970**, *103*, 2450–2462.
[44] R. Gigg, C. D. Warren, *J. Chem. Soc. C* **1968**, 1903–1911.
[45] J. J. Oltvoort, C. A. A. van Boeckel, J. H. de Koning, J. H. van Boom, *Synthesis* **1981**, 305–308.
[46] C. Gauthier, J. Legault, S. Lavoie, S. Rondeau, S. Tremblay, A. Pichette, *Tetrahedron* **2008**, *64*, 7386–7399.
[47] I. C. Sun, H.-K. Wang, Y. Kashiwada, J.-K. Shen, L. M. Cosentino, C.-H. Chen, L.-M. Yang, K.-H. Lee, *J. Med. Chem.* **1998**, *41*, 4648–4657.
[48] L. Voutquenne, C. Lavaud, G. Massiot, L. Le Men-Olivier, *Pharm. Biol.* **2002**, *40*, 253–262.
[49] N. S. Bhacca, D. Horton, H. Paulsen, *J. Org. Chem.* **1968**, *33*, 2484–2487.
[50] D. A. Horita, P. J. Hajduk, L. E. Lerner, *Glycoconjugate J.* **1997**, *14*, 691–696.
[51] K. Bock, I. Lundt, C. Pedersen, *Tetrahedron Lett.* **1973**, *14*, 1037–1040.
[52] K. Bock, C. Pedersen, *J. Chem. Soc. Perkin Trans. 2* **1974**, 293–297.
[53] K. Bock, C. Pedersen, *Acta Chem. Scand., Ser. B* **1975**, *29*, 258–264.
[54] P. Fügedi, in: *The Organic Chemistry of Sugars* (Eds.: D. E. Levy, P. Fügedi), CRC Press, Boca Raton, **2006**, chapter 4.
[55] R. J. Ferrier, P. C. Tyler, *J. Chem. Soc. Perkin Trans. 1* **1980**, 1528–1534.

Received: March 4, 2014
Published Online: ■

EurJOC European Journal of Organic Chemistry



A concise synthesis of 2,3,4,6-tetra-*O*-benzoyl-D-idopyranosyl trichloroacetimidate and lupane triterpenes bearing an unusual D-idopyranoside fragment is described. All new compounds were evaluated in vitro for their cytotoxic activities, and all exhibited activity in the micromolar range against human cancer cell lines.

P. Cmoch, A. Korda, L. Rárová, J. Oklešťková, M. Strnad,* K. Gwardiak, R. Karczewski, Z. Pakulski* 1–11

Synthesis of Lupane-Type Saponins Containing an Unusual α-D-Idopyranoside Fragment as Potent Cytotoxic Agents

Keywords: Carbohydrates / Glycoconjugates / Glycosylation / Steroids / Saponins / Drug discovery

