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# Synthesis, cytotoxicity and anti-inflammatory activity of rhamnose-containing ursolic and betulinic acid saponins†

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Betulinic acid and ursolic acid are ubiquitous, naturally-occurring triterpenoids exhibiting various pharmacological activities including cytotoxic and anti-inflammatory activities. However, these triterpenoids display unfavorable pharmacokinetic properties as well as low aqueous solubility. It has been shown that the presence of  $\alpha$ -L-rhamnose moieties positively modulates the anticancer activity of secondary metabolites. Herein we report the synthesis and *in vitro* evaluation of cytotoxic and anti-inflammatory activities of a series of rhamnose-containing ursolic and betulinic acid saponins. Relying on Schmidt's normal and inverse procedures, monorhamnosides, (1→4)-linked dirhamnosides as well as branched trirhamnosides and tetra-rhamnosides were synthesized in high yields with full control of stereoselectivity. A betulinic acid saponin bearing a 3-O- $\alpha$ -L-rhamnopyranosyl-(1→4)- $\alpha$ -L-rhamnopyranosyl residue was found to be a potent cytotoxic agent against human colorectal adenocarcinoma cells without damaging the healthy cells (selectivity ratio > 20) whereas rhamnose-containing ursolic acid saponins potently inhibited NO overproduction induced by LPS-stimulated macrophages. Our results reveal that rhamnose-containing ursolic and betulinic acid saponins represent promising therapeutic agents.

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## Introduction

Betulinic acid (BA, **1**) is a natural product-derived compound, and member of the lupane-type triterpenoid family. BA possesses multiple pharmacological activities including cytotoxic, anticancer, anti-inflammatory, antimalarial, and anti-HIV activities.<sup>1</sup> Because of its low *in vivo* toxicity, ubiquitous natural occurrence in the plant kingdom and broad spectrum of pharmacological activities, BA and its derivatives have been thoroughly investigated in the past few years.<sup>2–4</sup> Ursolic acid (UA, **2**) is another naturally occurring member of the triterpenoid family. UA is found in various plants, fruits, and medicinal herbs.<sup>5</sup> UA has been shown to possess a vast spectra of pharmacological activities including anti-metastatic, anti-angiogenic, antioxidant, anti-inflammatory, and antimicrobial activities.<sup>6</sup>

Although BA and UA are promising natural products from a medicinal point of view, their biopharmaceutical development has been hampered because of their low bioavailability and aqueous solubility.<sup>2,5</sup> In order to modulate the pharmacokinetic properties and increase their absorption by the organism, polar substituents have been added on the triterpenoid core by taking advantage of the presence of C-3 hydroxyl and C-28 carboxylic acid groups. For instance, C-28 amino acid,<sup>7</sup> C-3 phthalate,<sup>8</sup> and C-3 carbamate<sup>9</sup> as well as ionic derivatives<sup>10</sup> of BA have been synthesized and showed improved aqueous solubility and cytotoxic activities compared to BA. Recently, Baran and co-workers have developed a late-stage diversification approach for improving the pharmacokinetic properties of BA *via* C–H oxidation through a combination of chemical and enzymatic reactions.<sup>11</sup>

In parallel to these pioneering studies, we<sup>1,12–18</sup> and others<sup>19–23</sup> have been interested in coupling diverse hydro-soluble sugar moieties at the C-3 and/or C-28 positions of BA and other members of the lupane-type triterpenoid family such as betulin and lupeol. Among other things, we have shown that synthetic BA saponins bearing L-rhamnopyranose (Rha) residues at the C-3 position were potent cytotoxic agents<sup>15</sup> devoid of hemolytic activity<sup>12</sup> as compared to oleanane-type saponins. BA 3-O- $\alpha$ -L-rhamnopyranoside (**3**) exhibited higher cytotoxic activities against lung carcinoma (A549) and colorectal adenocarcinoma (DLD-1) than BA itself while being less active against human normal skin fibroblasts (WS1).<sup>15</sup> Furthermore, the

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anticancer activity of a bidesmosidic betulin saponin bearing Rha residues<sup>14</sup> was demonstrated *in vivo* against LLC1 tumor-bearing mice.<sup>24</sup> This compound was shown to induce apoptosis in cancer cells *via* disturbance of mitochondrial electron transfer chain, reduced reactive oxygen species, and decreased membrane potential.<sup>24</sup>

Although not fully understood, there seems to be a correlation between the presence of Rha residues and the anticancer properties of Rha-containing secondary metabolites. The intraperitoneal administration of Rha was shown to suppress cancer growth in mice.<sup>25</sup> Rhamnospicamycin, a Rha-containing analogue of the natural product spicamycin, was shown to be a potent cytotoxic agent against human myeloma cell lines with an IC<sub>50</sub> of 120 nM.<sup>26</sup> Rha-containing bufadienolides and cardenolides such as gamabufotalin rhamnoside and ouabain, respectively, are potent anticancer agents.<sup>27</sup> O'Doherty and co-workers reported the synthesis of Rha-containing digitoxin derivatives that showed excellent selectivity and activity against a panel of 60 human cancer cell lines.<sup>28</sup> Structure–activity relationship study highlighted that the  $\alpha$ -configuration of the glycosidic linkage as well as the presence of the L-enantiomer was a prerequisite to the anticancer activity of digitoxin glycosides.<sup>29</sup> Interestingly, Lou and co-workers have hypothesized that the presence of a Rha-binding lectins on human cells could play an important role in the anticancer activity of Rha-containing solasodine saponins.<sup>30</sup>

Within this framework, we report here the synthesis of a series of rhamnose-containing (mono, di, tri, and tetra) BA and UA saponins (compounds 3–10, Fig. 1). By designing these compounds, our objective was to significantly improve the aqueous solubility of BA and UA while preserving (or enhancing) their cytotoxic and anti-inflammatory activities through the multiple presentation of rhamnose units on the triterpenoid scaffold. The synthetic saponins were prepared from corresponding naturally occurring BA and UA, which are commercially available at low prices. The saponins were

synthesized following a regioselective approach aiming at minimizing the number of steps and protecting groups throughout the synthetic sequence. The Rha-containing saponins were evaluated for their cytotoxic and anti-inflammatory activities and some interesting selectivities were observed.

## Results and discussion

### Synthesis of BA saponins 3–6

Our work started with the synthesis of mono- and dirhamnoside-containing BA saponins (Scheme 1). Benzyl betulate (11)<sup>31</sup> was reacted with Schmidt 2,3,4-tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate donor (12)<sup>14</sup> under the promotion of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in anhydrous DCM providing rhamnoside 13 in 76% yield. Relying on the neighbouring participation of the C-2 benzoyl group, full control of stereoselectivity ( $\alpha$ -L-rhamnopyranoside) was obtained, as expected. Then, benzoyl groups were removed through Zemplén conditions to give triol 14. Debenzylation of the latter in the presence of catalytic amounts of 10% Pd/C in refluxing EtOAc led to the previously reported BA 3-*O*- $\alpha$ -L-rhamnopyranoside 3 with an improved overall yield (62% over three steps from derivative 11) without reducing the terminal alkene.<sup>15,32</sup>

Compound 14 was converted into the corresponding 2,3-di-*O*-isopropylidene derivative 15 following treatment with 2,2-dimethoxypropane (2,2-DMP) under the catalytic action of PTSA. The C-4' position of derivative 15 was then coupled with trichloroacetimidate (TCA) donor 12 to provide dirhamnoside 16 (72% yield) in exclusive  $\alpha$ -form. Cleavage of the isopropylidene group using 80% aqueous HOAc at 80 °C gave key intermediate 17 in 63% yield. Target BA dirhamnoside saponin 4 was obtained following hydrogenolysis and debenzoylation with 73% yield over two steps.

The synthesis of trirhamnoside BA saponin 5 was performed by taking advantage of derivative 17. As depicted in Scheme 2,

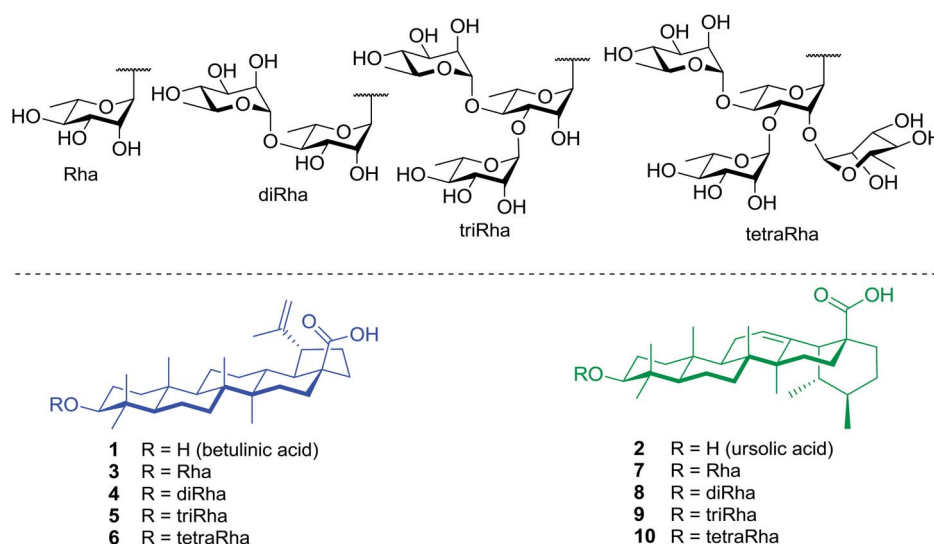
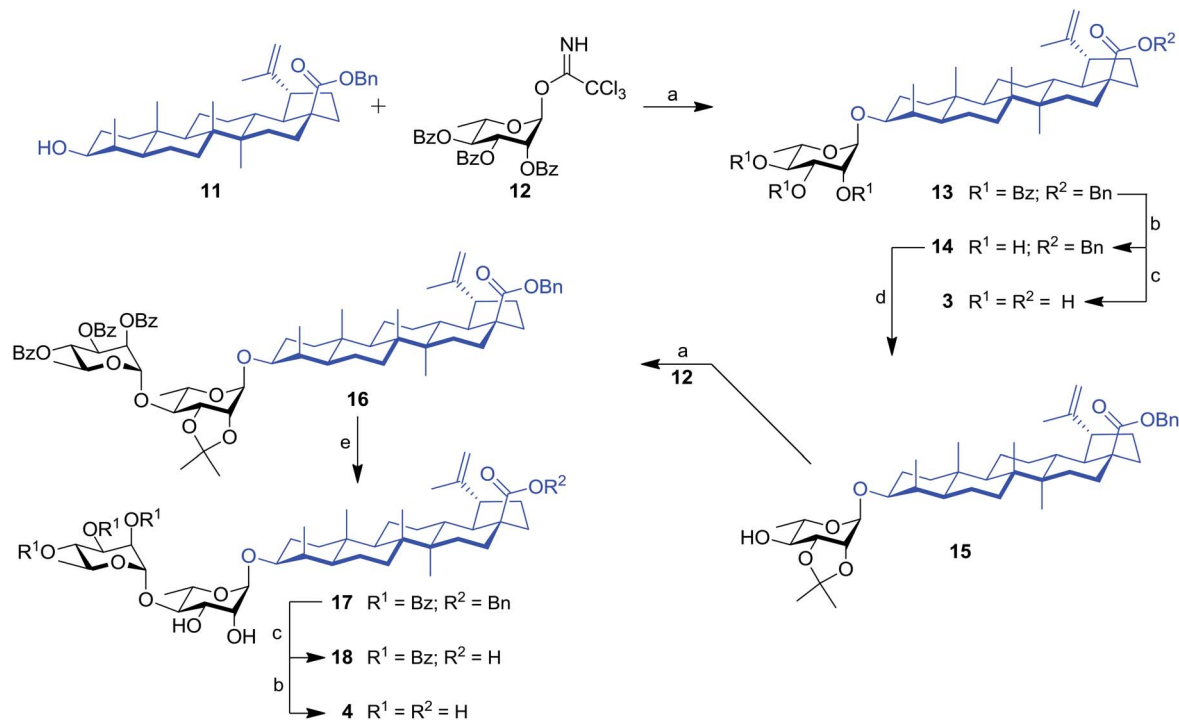


Fig. 1 Structures of target rhamnose-containing UA and BA saponins.



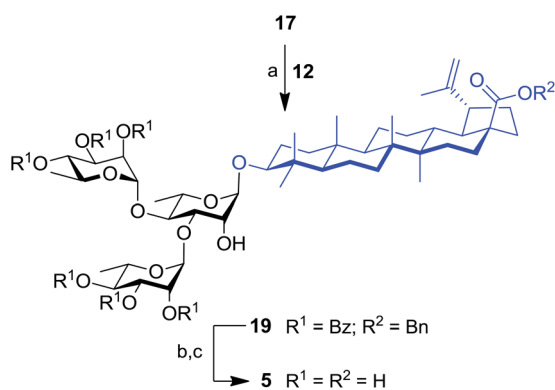


**Scheme 1** Synthesis of mono- and dirhamnoside-containing BA saponins (**3** and **4**). Reagents and conditions: (a) **12** (1.2–1.5 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM, 0 °C, 30 min, 76% (for **13**), 72% (for **16**); (b) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 97% (for **14**), 85% (for **4**); (c) 10% Pd/C, H<sub>2</sub>, EtOAc, 80 °C, 4 h, 86% (for **18**), 82% (for **3**, two steps from **13**); (d) 2,2-DMP (3 equiv.), PTSA (0.1 equiv.), acetone, rt, 2 h, 62%; (e) 80% aq. HOAc, DCM, rt, 16 h, 63%.

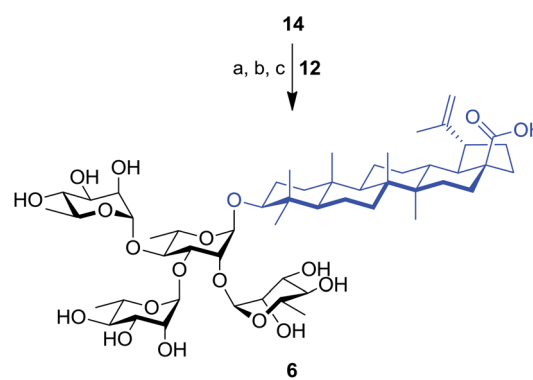
diol **17** was subjected to regioselective glycosylation<sup>33,34</sup> with TCA donor **12** affording C-3' and C-4' linked trirhamnoside **19** in 63% yield. The regioselectivity of the reaction at the C-3' position was confirmed *via* a 2D NMR HMBC experiment. A small amount of trirhamnoside at the C-2' position was also isolated at this step (data not shown). Cleavage of protecting groups in compound **19** led to target BA trirhamnoside saponin **5** in 68% yield over two steps.

The synthesis of tetra-rhamnoside **6** was our next target. Schmidt's inverse procedure (SIP)<sup>35</sup> that is known to minimize

donor degradation was preferred for this glycosylation in order to introduce three Rha residues in one step. Indeed, it was previously shown that multiple hydroxyl groups can be simultaneously glycosylated using SIP.<sup>17,36</sup> Therefore, as depicted in Scheme 3, triol **14** was subjected to glycosylation with 5.0 equiv. of donor **12** under SIP providing the expected tetra-rhamnoside in convenient yield (42%) with full control of stereoselectivity. The latter was deprotected using the aforementioned conditions. Purification using normal phase silica gel followed by solid phase extraction (SPE) furnished target saponin **6** in pure and homogeneous forms.



**Scheme 2** Synthesis of trirhamnoside-containing BA saponin (**5**). Reagents and conditions: (a) **12** (1.0 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM, 0 °C, 30 min, 63%; (b) 10% Pd/C, H<sub>2</sub>, EtOAc, 80 °C, 4 h; (c) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 68% (two steps).



**Scheme 3** Synthesis of tetra-rhamnoside-containing BA (**6**). Reagents and conditions: (a) inverse procedure, **12** (5.0 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM, -15 to 0 °C, 25 min; (b) 10% Pd/C, H<sub>2</sub>, EtOAc, 80 °C, 3 h; (c) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 37% (three steps).

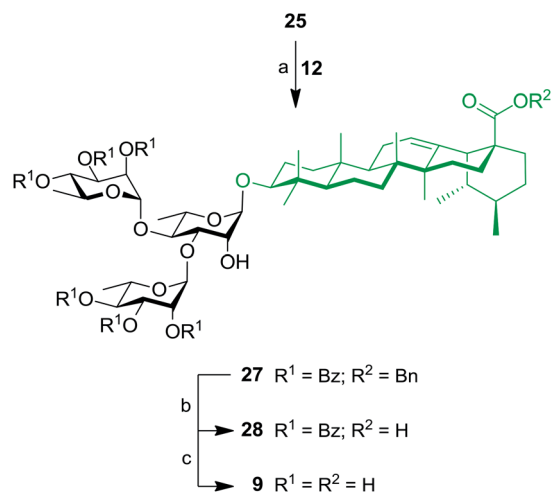


### Synthesis of UA saponins 7–10

These results prompted us to apply a similar synthetic approach to prepare rhamnose-containing UA saponins 7–10. Therefore, as shown in Scheme 4, coupling between benzyl ursolate (**20**)<sup>37</sup> and TCA donor **12** gave compound **21** with 75% yield. Removal of benzoyl groups through Zemplén deacylation gave triol **22**. Pd-catalyzed hydrogenolysis afforded UA 3-*O*- $\alpha$ -L-rhamnopyranoside (**7**) in 74% yield over two steps. Protection of triol **22** with an isopropylidene group afforded derivative **23**, which was rhamnosylated at the C-4' position providing dirhamnoside **24** in 70% yield. Cleavage of the isopropylidene group generated diol **25** (83% yield), which was subjected to global cleavage of protecting groups giving target dirhamnoside-containing UA saponin **8**.

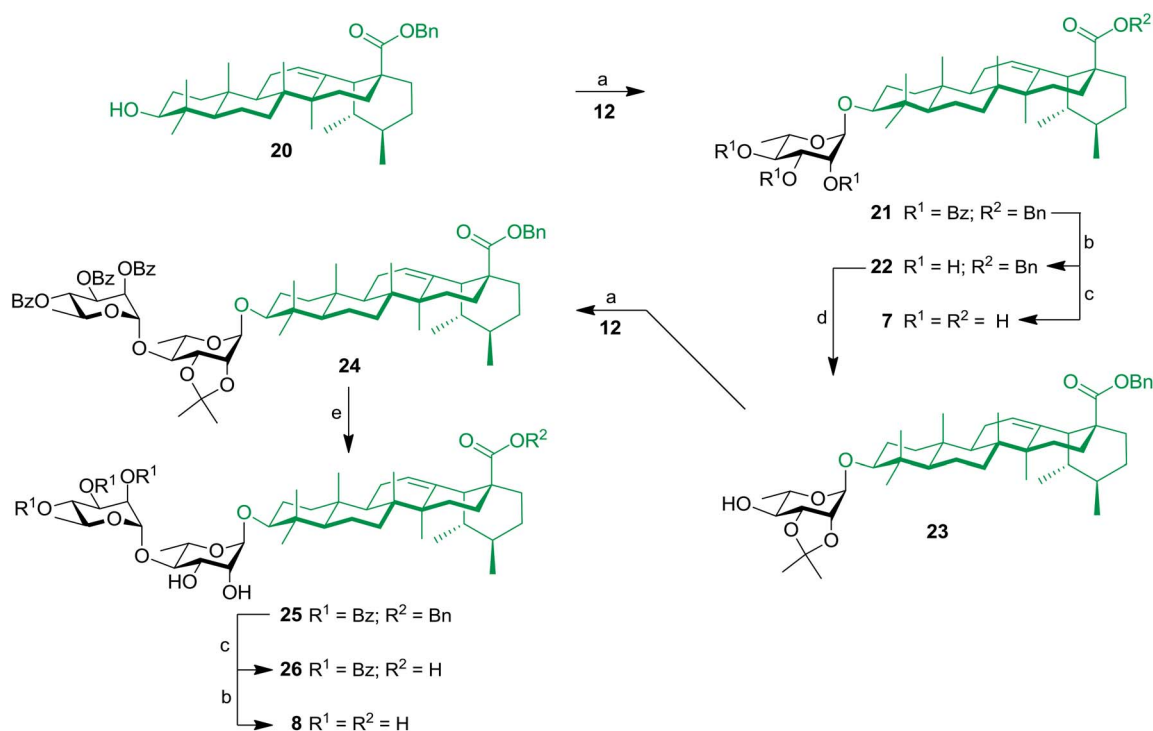
Thereafter, key intermediate **25** was engaged into regio-selective glycosylation with TCA donor **12** under TMSOTf promotion (Scheme 5) to give trirhamnoside **27** in 62% yield. The formation of the (1'''  $\rightarrow$  3') linkage was proved by a 2D NMR HMBC experiment, which showed strong cross-peaks from H-1''' to C-3'. Target trirhamnoside-containing UA saponin **9** was obtained following global deprotection using previously mentioned conditions in 68% yield over two steps.

At this stage, SIP was used for the simultaneous introduction of three Rha residues. As depicted in Scheme 6, triol **22** was reacted with 5.0 equiv. of TCA donor **12** to provide fully protected tetrarhamnoside **29** in a convenient 47% yield.



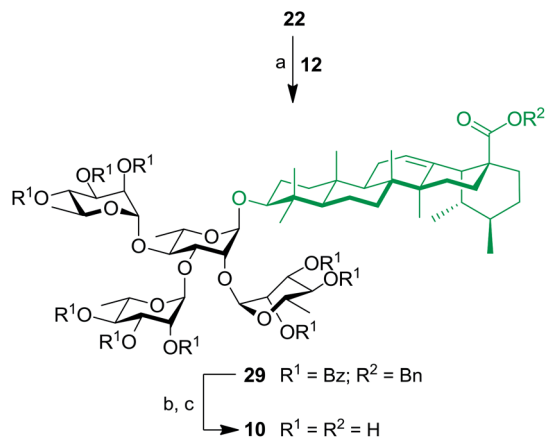
Scheme 5 Synthesis of trirhamnoside-containing UA saponin (**9**). Reagents and conditions: (a) **12** (1.1 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM, 0 °C, 10 min, 62%; (b) 10% Pd/C, H<sub>2</sub>, EtOAc, 80 °C, 4 h, 81%; (c) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 84%.

Deprotection of benzyl and benzoyl groups were performed to give target tetrarhamnoside-containing UA saponin **10** in 90% over two steps. All of the synthesized BA and UA saponins as well as parent triterpenoids were evaluated for their cytotoxic and anti-inflammatory activities.



Scheme 4 Synthesis of mono- and dirhamnoside-containing UA saponins (**7** and **8**). Reagents and conditions: (a) **12** (1.2 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM, 0 °C, 30 min, 75% (for **21**), 70% (for **24**); (b) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 98% (for **22**), 86% (for **8**); (c) 10% Pd/C, H<sub>2</sub>, EtOAc, 80 °C, 4 h, 89% (for **26**), 75% (for **7**, two steps from **21**); (d) 2,2-DMP (3 equiv.), PTSA (0.1 equiv.), CH<sub>3</sub>COCH<sub>3</sub>, rt, 2 h, 74%; (e) 80% aq. HOAc, DCM, rt, 16 h, 83%.





**Scheme 6** Synthesis of tetra-rhamnoside-containing UA saponin (**10**). Reagents and conditions: (a) inverse procedure, **12** (5.0 equiv.), TMSOTf (0.1 equiv.), 4 Å MS, DCM,  $-15$  to  $0$  °C, 25 min, 47%; (b) 10% Pd/C,  $H_2$ , EtOAc,  $80$  °C, 3 h; (c) NaOMe, MeOH/DCM 2 : 1, rt, 16 h, 90% (two steps).

### Cytotoxic and anti-inflammatory activities of triterpenes and saponins 1–10

The cytotoxicity of compounds **1–10** was evaluated against human colorectal adenocarcinoma (DLD-1) and healthy human skin fibroblasts (WS1), using Hoechst assay as described in the experimental procedure. The results presented in Table 1 are expressed as the concentration inhibiting 50% of cell growth ( $IC_{50}$ ). Rha-2-Bet, *i.e.*, 28-*O*- $\alpha$ -L-rhamnopyranosylbetulin 3 $\beta$ -*O*- $\alpha$ -L-rhamnopyranoside, was used as positive control with  $IC_{50}$  values of  $2.08$   $\mu$ M against DLD-1 and  $2.5$   $\mu$ M against WS-1.<sup>14</sup> BA (**1**) and UA (**2**) were found cytotoxic against cancer cells with  $IC_{50}$  respectively of  $20$  and  $13$   $\mu$ M as well as healthy cells with  $IC_{50}$  respectively of  $36$  and  $14$   $\mu$ M. As previously reported, the addition of a single rhamnose moiety to BA (**3**) at the C-3 position selectively improved cytotoxic activity toward cancer cells with  $IC_{50}$  of  $4$   $\mu$ M in comparison with  $33$   $\mu$ M for healthy cells.<sup>15</sup>

Interestingly, saponin **4** containing a dirhamnose grafted to BA at the same position inhibited cancer cell growth, DLD-1 ( $IC_{50} = 5$   $\mu$ M), without affecting healthy cells, WS1 ( $IC_{50} > 100$   $\mu$ M). In contrast, saponins **5** and **6** containing, respectively, a tri- or a tetra-rhamnose were inactive with  $IC_{50} > 100$   $\mu$ M. In the other hand, the addition of a single rhamnose (**7**) or a dirhamnose (**8**) at the C-3 position of UA did not improve the selectivity or the cytotoxic activity. As observed with BA, the presence of tri- (**9**) or tetra-rhamnose (**10**) residues inhibited the cytotoxicity of UA.

Anti-inflammatory activity of compounds **1–10** was also evaluated using LPS-stimulated RAW 264.7 macrophages, which induces NO overproduction. L-NAME was used as positive control with NO inhibition of 54% at a concentration of  $250$   $\mu$ M. Compounds **1**, **2**, and **7** inhibited NO overproduction induced by LPS with  $EC_{50}$  ranging from  $9$  to  $14$   $\mu$ M but macrophage cytotoxicity appeared at  $20$   $\mu$ M. Moreover, compounds **3–6** were found inactive with  $EC_{50} > 50$   $\mu$ M. In contrast, all of the UA rhamnosidic derivatives (**7–10**) inhibited NO overproduction with  $EC_{50}$  ranging from  $9.8$  to  $16$   $\mu$ M. Interestingly, the presence of mono- (**7**), di- (**8**), tri- (**9**) or tetra-rhamnosides (**10**) at the C-3 position of UA decreased gradually the cytotoxicity against healthy cells with  $IC_{50}$  respectively of  $23$   $\mu$ M,  $70$   $\mu$ M and  $>100$   $\mu$ M but retained the anti-inflammatory activity.

### Conclusions

In summary, we accomplished the straightforward synthesis of a series of rhamnose-containing saponins (**3–10**) using the bioactive, naturally-occurring BA (**1**) and UA (**2**) as aglycones. Monorhamnosides, (1 $\rightarrow$ 4)-linked dirhamnosides as well as branched trirhamnosides and tetra-rhamnosides were synthesized in high yields relying on both Schmidt's normal and inverse glycosylation procedures. Outstandingly, 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-betulinic acid (**4**) was found to exhibit potent cytotoxic activity against human colorectal adenocarcinoma cells without damaging the healthy cells (selectivity ratio  $> 20$ ) whereas UA saponins containing more than

**Table 1** Cytotoxic and anti-inflammatory activities of compounds **1–10**

Cpd	Cytotoxicity, $C_{50}^{a,c}$ ( $\mu$ M)		Anti-inflammatory activity, $EC_{50}^{b,c}$ ( $\mu$ M)
	DLD-1	WS-1	
<b>1</b>	$20 \pm 3$	$36 \pm 1$	$14 \pm 1$
<b>2</b>	$13 \pm 2$	$14 \pm 2$	$9 \pm 1$
<b>3</b>	$4.0 \pm 0.5$	$33 \pm 6$	NA <sup>d</sup>
<b>4</b>	$5 \pm 1$	$>100$	NA <sup>d</sup>
<b>5</b>	$>100$	$>100$	NA <sup>d</sup>
<b>6</b>	$>100$	$>100$	NA <sup>d</sup>
<b>7</b>	$15 \pm 1$	$23 \pm 1$	$10.5 \pm 0.3$
<b>8</b>	$>100$	$70 \pm 4$	$16 \pm 3$
<b>9</b>	$>100$	$>100$	$9.8 \pm 0.3$
<b>10</b>	$>100$	$>100$	$11 \pm 2$
Rha-2-Bet	$2.08 \pm 0.03$	$2.5 \pm 0.3$	$14 \pm 1$

<sup>a</sup>  $IC_{50}$ : concentration inhibiting 50% of cell growth. 28-*O*- $\alpha$ -L-Rhamnopyranosylbetulin 3 $\beta$ -*O*- $\alpha$ -L-rhamnopyranoside (Rha-2-Bet) was used as positive control. <sup>b</sup>  $EC_{50}$ : efficacy concentration inhibiting 50% of NO overproduction induced by LPS. L-NAME was used as positive control inhibits 54% of NO overproduction at  $250$   $\mu$ M. <sup>c</sup> The results are the mean  $\pm$  standard deviation of three determination and are representative of three different experiments. <sup>d</sup> NA: not active; compound was considered inactive when  $EC_{50}$  is  $\geq 50$   $\mu$ M.



one sugar unit were inactive against cancer cells. Moreover, we found that rhamnose-containing UA saponins 7–10 potently inhibited NO overproduction induced by LPS-stimulated macrophages. Collectively, our results suggest that the cytotoxic and anti-inflammatory activities of rhamnose-containing saponins are strongly modulated by both the nature of the triterpenes and the number and attachment of sugar units.

## Experimental section

### General methods

Air and water sensitive reactions (glycosylation especially) were performed under nitrogen or Ar atmosphere. Moisture sensitive reagents were introduced in reaction media *via* a dry syringe. AcroSeal® extra dry solvents over molecular sieves (dichloromethane, ethyl acetate, tetrahydrofuran and methanol) were purchased from Acros Organics. All commercial reagents were used without further purification. Purification of reaction products were carried out using 60–230 mesh silica gel obtained from Silicycle (Canada). Compounds were eluted with reagent grade solvent purchased from Fisher Scientific. Analytical thin-layer chromatography was performed with silica gel 60 F<sub>254</sub>, 0.25 mm pre-coated TLC plates, supplied by Silicycle (Canada), and visualized using UV light (254 nm) and cerium molybdate (2 g Ce(SO<sub>4</sub>)<sub>4</sub>(NH<sub>4</sub>)<sub>4</sub>, 5 g MoO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>, 200 mL H<sub>2</sub>O, and 20 mL H<sub>2</sub>SO<sub>4</sub>) with charring. All reaction yields are not optimized values. Optical rotations were measured on a Rudolph Research Analytical AUTOPOL IV digital polarimeter. Absorption UV spectra were recorded with an Agilent 8453 diode-array spectrophotometer. The 1D and 2D NMR spectra (<sup>1</sup>H–<sup>1</sup>H COSY, HSQC, HMBC and NOESY) were performed using an Avance 400 Bruker spectrometer (400.13 MHz for <sup>1</sup>H, 100.61 MHz for <sup>13</sup>C spectra) equipped with a 5 mm QNP-probe. All spectra were acquired in CDCl<sub>3</sub>, CD<sub>3</sub>OD, DMSO, or in a mixture of CDCl<sub>3</sub>–CD<sub>3</sub>OD 1 : 1. Chemical shifts are reported in ppm ( $\delta$ ) relative to TMS (0 ppm). The modulus of coupling constants ( $J$ ), extracted from the <sup>1</sup>H NMR spectrum, are reported in Hz. HPLC-APCI MS (negative mode) were obtained from an Agilent 1100 series system consisting of a degasser, a quaternary pump, an automatic injector, a temperature-controlled column compartment, a diode array detector and a mass selective detector Agilent G1946 VL model equipped with an APCI source. Analytical separations were performed at a flow rate of 1.0 mL min<sup>-1</sup> and a column temperature of 25 °C. Preparative HPLC separation (Agilent 1100) were carried out on a 20.0 × 250 mm C<sub>18</sub> column using a multiple wavelength detector and an automatic fraction collector. Chromatographic conditions were the following: gradient elution with H<sub>2</sub>O : CH<sub>3</sub>CN (10 → 100%) at flow rate of 20.0 mL min<sup>-1</sup>. Mass spectral data (HRMS) were obtained at NanoQAM University of Québec at Montréal (UQAM) and department of chemistry at University of Montréal (UdeM), Québec, Canada.

### General procedure for normal Schmidt glycosylation

Appropriate triterpene acceptor (1 equiv.) and trichloroacetimidate donor (1.5 equiv.) were premixed with 4 Å

molecular sieves in dry DCM (5 mL) under Ar atmosphere. After 30 min, TMSOTf (0.1 equiv.) was added dropwise under Ar while keeping rigorous anhydrous conditions. The reaction was generally stirred during 30 min, after which Et<sub>3</sub>N (0.2 equiv.) was added to quench the reaction. The suspension was filtered, and the solvents were evaporated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel.

### General procedure for inverse Schmidt glycosylation

To a stirred suspension of an appropriate triterpene acceptor and 4 Å molecular sieves in dry DCM (5 mL) was added TMSOTf (0.1 equiv.). The mixture was kept at –15 °C under rigorous anhydrous conditions. After 30 min, a solution of trichloroacetimidate donor (5 equiv.) in dry DCM was added to the mixture over 5 min. The mixture was stirred at –15 °C for 25 min, after which the reaction was quenched with Et<sub>3</sub>N (0.2 equiv.) and filtered. The filtrate was concentrated under reduced pressure to give a residue, which was purified by flash column chromatography.

### General procedure for removal of benzoyl groups

A freshly prepared solution of NaOMe in MeOH (0.5 M) was added to an appropriate benzoylated compound suspended in dry MeOH or a mixture of MeOH–DCM (2 : 1 v/v). The mixture was stirred overnight at rt. When TLC indicated the completion, the reaction was neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form) resin and filtered. The filtrate was concentrated to dryness and purified by silica gel flash chromatography.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-28-benzyl betulinate (13).** Based on the general procedure for direct Schmidt glycosylation, benzyl betulinatate **11** (ref. 31) (200 mg, 375  $\mu$ mol) and 2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate **12** (279 mg, 450  $\mu$ mol) was coupled in DCM (8 mL) with TMSOTf (7  $\mu$ L, 37.5  $\mu$ mol) as Lewis acid for 30 min. The reaction was then quenched with Et<sub>3</sub>N (22  $\mu$ L) and filtered. Purification by chromatography gave the expected compound **13** (371 mg, 76%) as colorless oil,  $R_f$  = 0.62 (7 : 3, hexanes–EtOAc), [ $\alpha$ ]<sub>D</sub><sup>20</sup> –15.9 (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (br d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.99 (br d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.83 (br d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.60 (br t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.51 (br t,  $J$  = 7.5 Hz, 1H, CH-Bz), 7.49 (br t,  $J$  = 7.5 Hz, 2H, CH-Bz), 7.45–7.30 (m, 7H, CH-Bz, CH-Bn), 7.26 (br t,  $J$  = 7.7 Hz, 3H, CH-Bz), 5.82 (dd,  $J$  = 10.1, 3.2 Hz, 1H, H-3'), 5.68 (t,  $J$  = 10.1 Hz, 1H, H-4'), 5.64 (dd,  $J$  = 3.1, 1.5 Hz, 1H, H-2'), 5.16 (d,  $J$  = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 5.09 (d,  $J$  = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 5.07 (br s, 1H, H-1'), 4.73 (br s, 1H, H-29a), 4.59 (br s, 1H, H-29b), 4.31 (dq,  $J$  = 10.1, 6.1 Hz, 1H, H-5'), 3.19 (t,  $J$  = 8.2 Hz, 1H, H-3), 3.03 (td,  $J$  = 10.8, 4.5 Hz, 1H, H-19), 2.29 (br d,  $J$  = 12.4 Hz, 1H, H-16a), 2.19 (td,  $J$  = 12.6, 3.1 Hz, 1H, H-13), 1.68 (s, 3H, H-30), 1.33 (d,  $J$  = 6.2 Hz, H-6'), 1.04 (s, 3H, H-23), 0.95 (s, 3H, H-27), 0.92 (s, 3H, H-24), 0.86 (s, 3H, H-25), 0.77 (s, 3H, H-26), 0.71 (br d,  $J$  = 8.6 Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C-28), [165.9, 165.7, 165.6 (CO-Bz)], 150.6 (C-20), 136.5 (C-Bn), [133.4, 133.3, 133.1 (CH-Bz)], [129.9 (2 $\times$ ), 129.8 (2 $\times$ ), 129.7 (2 $\times$ ) (CH-Bz)], [129.6, 129.4, 129.3 (C-Bz)], [128.6 (2 $\times$ ), 128.5



(2×), 128.4 (2×), 128.3 (4×), 128.1 (CH-Bz, CH-Bn)], 109.6 (C-29), 99.7 (C-1'), 90.1 (C-3), 72.0 (C-4'), 71.2 (C-2'), 70.2 (C-3'), 66.8 (C-5'), 65.7 (CH<sub>2</sub>-Bn), 56.6 (C-17), 55.5 (C-5), 50.5 (C-9), 49.4 (C-18), 46.9 (C-19), 42.4 (C-14), 40.7 (C-8), 39.1 (C-4), 38.7 (C-1), 38.2 (C-13), 36.9 (2×, C-22, C-10), 34.3 (C-7), 32.1 (C-16), 30.6 (C-21), 29.6 (C-15), 28.3 (C-23), 25.7 (C-2), 25.5 (C-12), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 17.6 (C-6'), 16.4 (C-24), 16.2 (C-25), 15.8 (C-26), 14.7 (C-27). HRMS calcd for C<sub>64</sub>H<sub>77</sub>O<sub>10</sub> [M + H]<sup>+</sup> 1005.5511, found 1005.5507; calcd for C<sub>64</sub>H<sub>76</sub>O<sub>10</sub>Na [M + Na]<sup>+</sup> 1022.5777, found 1022.5767.

**3-O- $\alpha$ -l-Rhamnopyranosyl-28-benzyl betulinic acid (14).** Based on the general procedure for benzoyl groups removal, compound **13** (230 mg, 232  $\mu$ mol) was dissolved in MeOH-DCM (2 : 1, 3 mL) with NaOMe in MeOH (0.5 M, 3 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (9 : 1, DCM-MeOH) to give compound **14** (153 mg, 97%) as colorless oil, R<sub>f</sub> = 0.5 (CHCl<sub>3</sub> : MeOH, 7 : 1), [ $\alpha$ ]<sub>D</sub><sup>20</sup> -18.9 (c 3, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.39–7.28 (m, 5H, H-Ar), 5.14 (d, J = 12.4 Hz, 1H, CH<sub>2</sub>-Bn), 5.10 (d, J = 12.4 Hz, 1H, CH<sub>2</sub>-Bn), 4.77 (s, 1H, H-1'), 4.73 (s, 1H, H-29a), 4.60 (s, 1H, H-29b), 3.90 (s, 1H, H-2'), 3.81–3.68 (m, 2H, H-3', H-5'), 3.44 (d, J = 9.9 Hz, 1H, H-4'), 3.08–2.97 (m, 2H, H-3, H-19), 2.28 (d, J = 10.4 Hz, 1H, H-16a), 2.18 (t, J = 11.1 Hz, 1H, H-13), 1.68 (s, 3H, H-30), 1.25 (d, J = 6.6 Hz, 3H, H-6'), 0.94 (s, 3H, H-27), 0.86 (s, 3H, H-23), 0.78 (s, 3H, H-25), 0.75 (s, 3H, H-26), 0.71 (s, 3H, H-24), 0.63 (br d, J = 7.3 Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C-28), 150.5 (C-20), 136.4 (C-Bn), [128.4 (2×), 128.2 (2×), 128.0 (CH-Bn)], 109.6 (C-29), 102.3 (C-1'), 89.3 (C-3), 72.8 (C-4'), 71.8 (C-3'), 71.3 (C-2'), 68.0 (C-5'), 65.7 (CH<sub>2</sub>-Bn), 56.5 (C-17), 55.4 (C-5), 50.5 (C-9), 49.4 (C-18), 46.9 (C-19), 42.3 (C-14), 40.6 (C-8), 39.0 (C-4), 38.6 (C-1), 38.1 (C-13), 36.9 (C-22), 36.8 (C-10), 34.2 (C-7), 32.1 (C-16), 30.5 (C-21), 29.5 (C-15), 28.1 (C-23), 25.5 (C-12), 25.4 (C-2), 20.8 (C-11), 19.3 (C-30), 18.2 (C-6), 17.4 (C-6'), 16.2 (C-24), 16.1 (C-25), 15.8 (C-26), 14.6 (C-27). HRMS calcd for C<sub>43</sub>H<sub>65</sub>O<sub>7</sub> [M + H]<sup>+</sup> 693.4725, found 693.4698.

**3-O- $\alpha$ -l-Rhamnopyranosyl betulinic acid (3).** In parallel, BA 3-O- $\alpha$ -l-rhamnopyranoside **3** was synthesized in two steps starting from **13**. A solution of **13** (50 mg, 50  $\mu$ mol) and 10% Pd/C (1 mg) in dry EtOAc (3 mL) was refluxed and purged with H<sub>2</sub> for 2 h. When TLC indicated the disappearance of the starting material, the residue was filtered over Celite® and concentrated before the removal of benzoyl groups. This was accomplished according to the general procedure using MeOH-DCM 2 : 1 as solvent. The reaction was neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (7 : 1, DCM-MeOH) to give compound **3** (25 mg, 82%, two steps) as colorless oil, R<sub>f</sub> = 0.3 (DCM : MeOH, 7 : 1). The NMR data are in adequation with those described in the literature.<sup>15,32</sup>

**3-O-(2,3-Di-O-isopropylidene- $\alpha$ -l-rhamnopyranosyl)-28-benzyl betulinic acid (15).** Compound **14** (140 mg, 206  $\mu$ mol) was dissolved in acetone (5 mL) to which was added 2,2-dimethoxypropane (76  $\mu$ L, 618  $\mu$ mol) and PTSA (3.55 mg, 20  $\mu$ mol) as catalyst. After 2 h, reaction was evaporated to dryness and purified by flash chromatography (7 : 3, hexanes-EtOAc) to give compound **15** (92 mg, 62%) as colorless oil, R<sub>f</sub> = 0.7 (hexanes-EtOAc, 7 : 3), [ $\alpha$ ]<sub>D</sub><sup>20</sup> -5.8 (c 2.3, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.42–7.22

(m, 5H, H-Ar), 5.15 (d, J = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 5.09 (d, J = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 4.97 (s, 1H, H-1'), 4.72 (br s, 1H, H-29a), 4.59 (br s, 1H, H-29b), 4.18–4.09 (m, 2H, H-2', H-3'), 3.83 (dq, J = 8.8, 6.3 Hz, 1H, H-5'), 3.42 (dd, J = 8.4, 6.3 Hz, 1H, H-4'), 3.11 (dd, J = 11.4, 4.5 Hz, 1H, H-3), 3.02 (td, J = 10.6, 4.2 Hz, 1H, H-19), 2.27 (br d, J = 12.2 Hz, 1H, H-16a), 2.17 (td, J = 12.7, 3.1 Hz, 1H, H-13), 1.68 (s, 3H, H-30), 1.52 (s, 3H, CH<sub>3</sub>-iso), 1.37 (s, 3H, CH<sub>3</sub>-iso), 1.27 (d, J = 6.4 Hz, 3H, H-6'), 0.93 (s, 3H, H-27), 0.91 (s, 3H, H-23), 0.80 (s, 3H, H-25), 0.75 (s, 6H, H-24, H-26), 0.67 (br d, J = 9.6 Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C-28), 150.6 (C-20), 136.5 (C-Bn), [128.5 (2×), 128.2 (2×), 128.1 (CH-Bn)], 109.6 (C-29), 109.4 (C-iso), 99.8 (C-1'), 89.1 (C-3), 78.1 (C-3'), 75.8 (C-2'), 74.0 (C-4'), 66.5 (C-5'), 65.7 (CH<sub>2</sub>-Bn), 56.5 (C-17), 55.5 (C-5), 50.5 (C-9), 49.4 (C-18), 46.9 (C-19), 42.4 (C-14), 40.6 (C-8), 39.1 (C-4), 38.7 (C-1), 38.2 (C-13), 36.9 (C-22), 36.9 (C-10), 34.2 (C-7), 32.1 (C-16), 30.6 (C-21), 29.5 (C-15), 28.2 (C-23), 27.9 (CH<sub>3</sub>-iso), 26.1 (CH<sub>3</sub>-iso), 25.6 (C-12), 25.5 (C-2), 20.9 (C-11), 19.4 (C-30), 18.2 (C-6), 17.7 (C-6'), 16.3 (C-24), 16.2 (C-25), 15.8 (C-26), 14.7 (C-27). HRMS calcd for C<sub>46</sub>H<sub>69</sub>O<sub>7</sub> [M + H]<sup>+</sup> 733.5038, found 733.5010.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -l-rhamnopyranosyl-(1→4)-2,3-di-O-isopropylidene- $\alpha$ -l-rhamnopyranosyl)-28-benzyl betulinic acid (16).** Based on the general procedure for normal Schmidt glycosylation, compound **15** (62 mg, 86  $\mu$ mol) and 2,3,4-tri-O-benzoyl- $\alpha$ -l-rhamnopyranosyl trichloroacetimidate **12** (63 mg, 103  $\mu$ mol) was coupled in DCM (4 mL) with TMSOTf (1.5  $\mu$ L, 9  $\mu$ mol) as Lewis acid for 30 min. When TLC indicated completion, reaction was then quenched with Et<sub>3</sub>N (5  $\mu$ L) filtered and evaporated before purification by flash chromatography (8 : 2, hexanes-EtOAc) to give compound **16** (73 mg, 72%) as colorless oil, R<sub>f</sub> = 0.6 (hexanes-EtOAc, 8 : 2), [ $\alpha$ ]<sub>D</sub><sup>20</sup> +35 (c 2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (br d, J = 7.7 Hz, 2H, CH-Bz), 7.96 (br d, J = 7.9 Hz, 2H, CH-Bz), 7.80 (br d, J = 8.0 Hz, 2H, CH-Bz), 7.61 (br t, J = 7.6 Hz, 1H, CH-Bz) 7.55–7.22 (m, 13H, CH-Bz, CH-Bn), 5.77 (dd, J = 10.0, 3.3 Hz, 1H, H-4''), 5.73 (dd, J = 3.2, 1.7 Hz, 1H, H-2''), 5.67 (t, J = 9.8 Hz, 1H, H-4''), 5.58 (d, J = 1.4 Hz, 1H, H-1''), 5.16 (d, J = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 5.10 (d, J = 12.3 Hz, 1H, CH<sub>2</sub>-Bn), 5.03 (s, 1H, H-1'), 4.74 (d, J = 2.4 Hz, 1H, H-29a), 4.61 (s, 1H, H-29b), 4.31 (dd, J = 7.2, 5.4 Hz, 1H, H-3'), 4.19 (dq, J = 9.5, 6.2 Hz, 1H, H-5''), 4.13 (d, J = 5.5 Hz, 1H, H-2'), 3.97 (dq, J = 10.0, 6.2 Hz, 1H, H-5'), 3.61 (dd, J = 9.9, 7.3 Hz, 1H, H-4'), 3.14 (dd, J = 9.9, 6.1 Hz, 1H, H-3), 3.03 (td, J = 10.7, 4.3 Hz, 1H, H-19), 2.29 (br d, J = 12.3 Hz, 1H, H-16a), 2.19 (td, J = 12.4, 3.3 Hz, 1H, H-13), 1.69 (s, 3H, H-29), 1.55 (s, 3H, CH<sub>3</sub>-iso), 1.38 (d, J = 5.8 Hz, 3H, H-6'), 1.37 (d, J = 6.0 Hz, 3H, H-6''), 1.35 (s, 3H, CH<sub>3</sub>-iso), 0.95 (s, 3H, H-27), 0.93 (s, 3H, H-23), 0.84 (s, 3H, H-25), 0.80 (s, 3H, H-24), 0.77 (s, 3H, H-26), 0.70 (br d, J = 8.5 Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C-28), [165.8, 165.6, 165.4 (CO-Bz)], 150.6 (C-20), 136.5 (C-Bz), [133.4, 133.3, 133.1, 130.0 (2×), 129.7 (2×), 129.7 (2×) (CH-Bz)], [129.5, 129.3, 129.2 (C-Bz)], 128.5<sub>3</sub> (2×), 128.4<sub>9</sub> (2×), 128.4<sub>1</sub> (2×), 128.3 (4×), 128.1 (CH-Bz, CH-Bn), 109.6 (C-29), 109.5 (C-iso), 99.5 (C-1'), 96.0 (C-1''), 89.0 (C-3), 78.2 (C-3'), 78.0 (C-4'), 76.3 (C-2'), 71.7 (C-4''), 70.8 (C-2''), 70.0 (C-3''), 67.3 (C-5''), 65.7 (CH<sub>2</sub>-Bn), 63.7 (C-5'), 56.6 (C-17), 55.5 (C-5), 50.6 (C-9), 49.5 (C-18), 46.9 (C-19), 42.4 (C-14), 40.7 (C-8), 39.2 (C-4), 38.8 (C-1), 38.2 (C-13), 36.9 (C-22), 36.9 (C-10), 34.3 (C-7), 32.1 (C-16), 30.6 (C-21), 29.6 (C-



15), 28.3 (C-23), 28.0 ( $CH_3$ -iso), 26.5 ( $CH_3$ -iso), 25.6 (C-12), 25.5 (C-2), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 18.1 (C-6''), 17.7 (C-6'), 16.3 (C-24), 16.2 (C-25), 15.8 (C-26), 14.7 (C-27). HRMS calcd for  $C_{73}H_{94}O_{14}N [M + NH_4]^+$  1208.6669, found 1208.6703.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl)-28-benzyl betulinic acid (17).** To a solution of compound **16** (197 mg, 167  $\mu$ mol) in DCM (5 mL) was added HOAc 80% aq. (15 mL). After 3 h stirring at 80  $^{\circ}$ C, reaction was evaporated to dryness and the crude was purified by flash chromatography (8 : 2, hexanes-EtOAc) to give compound **17** (120 mg, 63%) as colorless oil,  $R_f$  = 0.5 (hexanes-EtOAc, 8 : 2),  $[\alpha]_D^{20}$  +36.4 (*c* 2,  $CHCl_3$ ).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.07 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.97 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.83 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.59 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.52 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.49–7.22 (m, 12H, CH-Bz), 5.81 (dd,  $J$  = 10.1, 3.4 Hz, 1H, H-3''), 5.77 (dd,  $J$  = 3.2, 1.8 Hz, 1H, H-2''), 5.68 (t,  $J$  = 9.9 Hz, 1H, H-4''), 5.48 (br s, 1H, H-1''), 5.16 (d,  $J$  = 12.3 Hz, 1H,  $CH_2$ -Bn), 5.10 (d,  $J$  = 12.3 Hz, 1H,  $CH_2$ -Bn), 4.85 (s, 1H, H-1'), 4.73 (d,  $J$  = 2.4 Hz, 1H, H-29a), 4.60 (br s, 1H, H-29b), 4.29 (dq,  $J$  = 9.6, 6.2 Hz, 1H, H-5''), 4.07 (dd,  $J$  = 9.1, 3.3 Hz, 1H, H-3'), 4.01–3.90 (m, 2H, H-2', H-5'), 3.63 (t,  $J$  = 9.3 Hz, 1H, H-4'), 3.08 (dd,  $J$  = 10.8, 5.3 Hz, 1H, H-3), 3.03 (dd,  $J$  = 10.8, 4.4 Hz, 2H, H-19), 2.29 (br d,  $J$  = 12.3 Hz, 1H, H-16a), 2.19 (td,  $J$  = 12.8, 3.4 Hz, 1H, H-13), 1.69 (s, 3H, H-30), 1.37 (d,  $J$  = 6.8 Hz, 3H, H-6'), 1.36 (d,  $J$  = 6.6 Hz, 3H, H-6''), 0.95 (s, 3H, H-27), 0.89 (s, 3H, H-23), 0.84 (s, 3H, H-25), 0.79 (s, 3H, H-24), 0.76 (s, 3H, H-26), 0.66 (br d,  $J$  = 9.1 Hz, 1H, H-5).  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  175.8 (C-28), [165.9, 165.8, 165.8 (CO-Bz)], 150.6 (C-20), 136.5 (C-Bn), [133.5, 133.3, 133.2 (CH-Bz)], [129.9 (2 $\times$ ), 129.7 (4 $\times$ ) (CH-Bz)], [129.3, 129.3, 129.1 (C-Bz)], 128.6 (2 $\times$ ), 128.5 (2 $\times$ ), 128.4 (2 $\times$ ), 128.3<sub>1</sub> (2 $\times$ ), 128.2<sub>5</sub> (2 $\times$ ), 128.1 (CH-Bz, CH-Bn), 109.6 (C-29), 101.9 (C-1'), 98.9 (C-1''), 89.6 (C-3), 81.6 (C-4'), 71.9 (C-2'), 71.9 (C-3'), 71.6 (C-4''), 71.2 (C-2''), 70.1 (C-3''), 67.5 (C-5''), 66.2 (C-5'), 65.7 ( $CH_2$ -Bn), 56.5 (C-17), 55.5 (C-5), 50.5 (C-9), 49.4 (C-18), 46.9 (C-19), 42.4 (C-14), 40.6 (C-8), 39.1 (C-4), 38.7 (C-1), 38.2 (C-13), 36.9 (C-22), 36.9 (C-10), 34.3 (C-7), 32.1 (C-16), 30.6 (C-21), 29.6 (C-15), 28.2 (C-23), 25.6 (C-12), 25.5 (C-2), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 17.9 (C-6'), 17.6 (C-6''), 16.3 (C-24), 16.1 (C-25), 15.8 (C-26), 14.7 (C-27). HRMS calcd for  $C_{70}H_{90}O_{14}N [M + NH_4]^+$  1168.63558, found 1168.63697.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl) betulinic acid (18).** A solution of compound **17** (116 mg, 102  $\mu$ mol) and 10% Pd/C (2 mg) in dry EtOAc (5 mL) was refluxed and purged with  $H_2$ . When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite $^{\circ}$  and concentrated under reduced pressure. The residue was purified by flash chromatography (7 : 3, hexanes-EtOAc) to give compound **18** (92 mg, 86%) as colorless oil,  $R_f$  = 0.2 (hexanes-EtOAc, 7 : 3),  $[\alpha]_D^{20}$  +28.3 (*c* 2.2,  $CHCl_3$ ).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.07 (d,  $J$  = 8.2 Hz, 2H, CH-Bz), 7.97 (d,  $J$  = 8.3 Hz, 2H, CH-Bz), 7.82 (d,  $J$  = 8.2 Hz, 2H, CH-Bz), 7.62–7.20 (m, 12H, CH-Bz), 5.81 (dd,  $J$  = 10.2, 3.2 Hz, 1H, H-3''), 5.77–5.73 (m, 1H, H-2''), 5.68 (t,  $J$  = 9.9 Hz, 1H, H-4''), 5.48 (s, 1H, H-1''), 4.86 (s, 1H, H-1'), 4.75 (br s, 1H, H-29a), 4.62 (br s, 1H, H-29b), 4.29 (dq,  $J$  = 9.3, 6.1 Hz, 1H, H-5''), 4.10–4.05 (m, 1H, H-3'), 4.01–3.92 (m, 2H, H-2', H-5'), 3.64 (t,  $J$  = 9.2 Hz, 1H, H-4'), 3.10 (dd,  $J$  = 10.4, 5.2 Hz, 1H, H-3), 3.06–2.96 (m, 1H, H-19), 2.32–2.14 (m,

2H, H-16a, H-13), 1.70 (s, 3H, H-30), 1.38 (d,  $J$  = 6.6 Hz, 3H, H-6'), 1.36 (d,  $J$  = 6.6 Hz, 3H, H-6''), 0.98 (s, 3H, H-27), 0.94 (s, 3H, H-26), 0.91 (s, 3H, H-23), 0.87 (s, 3H, H-25), 0.81 (s, 3H, H-24), 0.70 (d,  $J$  = 8.5 Hz, 1H, H-5).  $^{13}C$  NMR (101 MHz,  $CDCl_3$ )  $\delta$  181.3 (C-28), [165.9 (2 $\times$ ), 165.8 (CO-Bz)], 150.4 (C-20), [133.5, 133.4, 133.2, 130.0, 129.7 (2 $\times$ ) (CH-Bz)], [129.2<sub>9</sub>, 129.2<sub>7</sub>, 129.1 (C-Bz)], [128.6, 128.4, 128.3 (CH-Bz)], 109.7 (C-29), 101.9 (C-1'), 98.9 (C-1''), 89.6 (C-3), 81.4 (C-4'), 71.9 (2 $\times$ , C-3', C-2'), 71.6 (C-4''), 71.2 (C-2''), 70.0 (C-3''), 67.6 (C-5''), 66.2 (C-5'), 56.4 (C-17), 55.5 (C-5), 50.5 (C-9), 49.3 (C-18), 46.9 (C-19), 42.4 (C-14), 40.7 (C-8), 39.1 (C-4), 38.7 (C-1), 38.4 (C-13), 37.1 (C-22), 36.9 (C-10), 34.3 (C-7), 32.2 (C-16), 30.6 (C-21), 29.7 (C-15), 28.2 (C-23), 25.6 (C-12), 25.5 (C-2), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 17.9 (C-6'), 17.6 (C-6''), 16.4 (C-24), 16.2 (C-25), 16.0 (C-26), 14.7 (C-27). HRMS calcd for  $C_{63}H_{84}O_{14}N [M + NH_4]^+$  1078.5856, found 1078.5901.

**3-O-( $\alpha$ -L-Rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl) betulinic acid (4).** Based on the general procedure for benzoyl groups removal, compound **18** (70 mg, 67  $\mu$ mol) was dissolved in MeOH-DCM (2 : 1, 3 mL) with NaOMe in MeOH (0.5 M, 3 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 ( $H^+$  form), filtered and purified by flash chromatography (7 : 1, DCM-MeOH) to give compound **4** (42 mg, 85%) as colorless oil,  $R_f$  = 0.4 ( $CHCl_3$  : MeOH, 7 : 1),  $[\alpha]_D^{20}$  –18.9 (*c* 3,  $CHCl_3$ ).  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.11 (br s, 1H, COOH), 5.06 (s, 1H, H-1''), 4.95 (s, 1H, OH), 4.76 (br s, 2H, OH), 4.69 (s, 1H, H-29a), 4.65 (br s, 1H, OH), 4.60–4.54 (m, 2H, H-1', H-29b), 3.69 (s, 1H, H-2''), 3.60 (s, 1H, H-2'), 3.58–3.51 (m, 2H, H-5', H-3'), 3.51–3.44 (m, 1H, H-5''), 3.43–3.30 (m, 2H, H-4', H-3''), 3.23–3.14 (m, 1H, H-4''), 3.04–2.89 (m, 2H, H-3, H-19), 2.23 (br t,  $J$  = 10.6 Hz, 1H, H-13), 2.11 (br d,  $J$  = 7.9 Hz, 1H, H-16a), 1.64 (s, 3H, H-30), 1.12 (d,  $J$  = 6.0 Hz, H-6'), 1.11 (d,  $J$  = 6.0 Hz, H-6''), 0.93 (s, 3H, H-27), 0.86 (s, 6H, H-23, H-26), 0.78 (s, 3H, H-25), 0.70 (s, 3H, H-24).  $^{13}C$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  177.2 (C-28), 150.2 (C-20), 109.5 (C-29), 102.5 (C-1'), 101.0 (C-1), 87.6 (C-3), 77.9 (C-4'), 71.8 (C-4''), 71.5 (C-3'), 71.1 (C-2'), 70.6 (C-3''), 70.5 (C-2''), 68.8 (C-5''), 66.6 (C-5'), 55.3 (C-17), 54.6 (C-5), 49.7 (C-9), 48.4 (C-18), 46.5 (C-19), 41.9 (C-14), 40.1 (C-8), 38.5 (C-4), 38.0 (C-1), 37.4 (C-13), 36.4 (C-10), 36.2 (C-22), 33.7 (C-7), 31.6 (C-16), 30.0 (C-21), 29.1 (C-15), 27.7 (C-23), 25.0 (2 $\times$ , C-2, C-12), 20.3 (C-11), 18.8 (C-30), 18.0 (C-6'), 17.7<sub>3</sub> (C-6), 17.6<sub>8</sub> (C-6''), 16.1 (C-24), 15.8 (C-25), 15.6 (C-26), 14.3 (C-27). HRMS calcd for  $C_{42}H_{72}O_{11}N [M + NH_4]^+$  766.51, found 766.5128.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl)-28-benzyl betulinic acid (19).** Based on the general procedure for normal Schmidt glycosylation, compound **17** (120 mg, 106  $\mu$ mol) and 2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate **12** (66 mg, 106  $\mu$ mol) was coupled in DCM (6 mL) with TMSOTf (2  $\mu$ L, 11  $\mu$ mol) as Lewis acid for 30 min. When TLC indicated completion, reaction was then quenched with  $Et_3N$  (5  $\mu$ L) filtered and evaporated before purification by flash chromatography (7 : 3, hexanes-EtOAc) to give compound **19** (107 mg, 63%) as colorless oil,  $R_f$  = 0.5 (hexanes-EtOAc, 7 : 3),  $[\alpha]_D^{20}$  +44.5 (*c* 1.9,  $CHCl_3$ ).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.02–7.74 (m, 12H, CH-Bz), 7.55–7.06 (m, 17H, CH-Bz, CH-Bn), 6.04 (dd,  $J$  = 3.4, 1.7 Hz, 1H, H-2''), 5.99 (dd,  $J$  = 10.1, 3.2 Hz, 1H, H-3''), 5.95 (dd,  $J$  = 10.2, 3.1 Hz, 1H, H-3''), 5.83 (dd,  $J$  = 3.5, 1.8 Hz, 1H,



H-2''), 5.75 (t,  $J = 10.0$  Hz, 1H, H-4''), 5.67 (t,  $J = 9.9$  Hz, 1H, H-4'''), 5.54 (d,  $J = 1.9$  Hz, 1H, H-1''), 5.37 (br s, 1H, H-1'''), 5.16 (d,  $J = 12.3$  Hz, 1H, CH<sub>2</sub>-Bn), 5.09 (d,  $J = 12.3$  Hz, 1H, CH<sub>2</sub>-Bn), 4.86 (s, 1H, H-1'), 4.73 (d,  $J = 2.5$  Hz, 1H, H-29a), 4.60 (br s, 1H, H-29b), 4.46 (dq,  $J = 9.7, 6.2$  Hz, 1H, H-5'''), 4.32 (dq,  $J = 9.6, 6.3$  Hz, 1H, H-5''), 4.22–4.14 (m, 2H, H-2', H-3'), 4.03 (dq,  $J = 9.3, 6.3$  Hz, 1H, H-5'), 3.84 (t,  $J = 9.2$  Hz, 1H, H-4'), 3.15–3.07 (m, 1H, H-3), 3.03 (td,  $J = 10.9, 4.5$  Hz, 1H, H-19), 2.29 (br d,  $J = 12.2$  Hz, 1H), 2.18 (td,  $J = 12.4, 3.2$  Hz, 1H), 1.68 (s, 3H, H-30), 1.43 (d,  $J = 6.2$  Hz, 3H, H-6'), 1.36 (d,  $J = 6.3$  Hz, 3H, H-6''), 1.35 (d,  $J = 6.2$  Hz, 3H, H-6'''), 0.94 (s, 3H, H-27), 0.91 (s, 3H, H-23), 0.86 (s, 3H, H-25), 0.83 (s, 3H, H-24), 0.76 (s, 3H, H-26), 0.68 (br d,  $J = 8.2$  Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C-28), [165.9, 165.8, 165.6, 165.5, 165.1, 164.9 (CO-Bz)], 150.6 (C-20), 136.5 (C-Bn), [133.2<sub>4</sub>, 133.2<sub>3</sub>, 133.1, 132.8, 132.7, 132.6, 129.9, 129.8<sub>2</sub>, 129.7<sub>7</sub>, 129.6<sub>3</sub>, 129.5<sub>9</sub>, 129.5<sub>1</sub>, 129.4, 129.3, 128.5, 128.4, 128.3<sub>5</sub>, 128.3<sub>3</sub>, 128.2<sub>7</sub>, 128.2<sub>1</sub>, 128.1<sub>0</sub>, 128.0<sub>8</sub>, 128.0 (CH-Bz, CH-Bn)], 109.6 (C-29), 101.8 (C-1'), 99.7 (C-1''), 99.5 (C-1'''), 89.9 (C-3), 81.7 (C-3'), 81.0 (C-4'), 72.0 (C-4'''), 72.0 (C-4''), 71.9 (C-2''), 71.3 (C-2'''), 71.1 (C-2'), 69.4 (C-3'''), 69.4 (C-3''), 67.7 (C-5'''), 67.6 (C-5''), 67.1 (C-5'), 65.7 (C-7), 56.6 (C-17), 55.5 (C-5), 50.5 (C-9), 49.5 (C-18), 47.0 (C-19), 42.4 (C-14), 40.7 (C-8), 39.1 (C-4), 38.7 (C-1), 38.2 (C-13), 36.9 (C-22), 36.9 (C-10), 34.3 (C-7), 32.1 (C-16), 30.6 (C-21), 29.7 (C-15), 29.6 (C-15), 28.3 (C-23), 25.7 (C-12), 25.5 (C-2), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6'), 18.3 (C-6), 17.7 (C-6''), 17.6 (C-6'''), 16.5 (C-24), 16.2 (C-25), 15.8 (C-26), 14.7 (C-27). HRMS calcd for C<sub>97</sub>H<sub>112</sub>O<sub>21</sub>N [M + NH<sub>4</sub>]<sup>+</sup> 1626.7721, found 1626.7762.

**3-O- $\alpha$ -L-Rhamnopyranosyl-(1  $\rightarrow$  2)-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  4)]- $\alpha$ -L-rhamnopyranosyl betulinic acid (5).** A solution of compound **19** (43 mg, 27  $\mu$ mol) and 10% Pd/C (1 mg) in dry EtOAc (3 mL) was refluxed and purged with H<sub>2</sub>. When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite® and concentrated under reduced pressure. The residue was dissolved in MeOH–DCM (2 : 1, 1.5 mL) with NaOMe in MeOH (0.5 M, 1.5 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (7 : 1, CHCl<sub>3</sub>–MeOH to 50 : 10 : 1, CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O) to give compound **5** (19.5 mg, 84%) as amorphous powder,  $R_f = 0.3$  (26 : 14 : 3 CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O), [ $\alpha$ ]<sub>D</sub><sup>20</sup> –6.42 (c, 1.09, 1 : 1, CHCl<sub>3</sub>–MeOH). <sup>1</sup>H NMR [400 MHz, DMSO-*d*<sub>6</sub> + 1 drop H<sub>2</sub>O]  $\delta$  4.82 (s, 1H, H-1''), 4.68 (m, 1H, H-29), 4.60 (s, 1H, H-1'''), 4.58–4.53 (s, 1H, H-1', H-29b), 3.75–3.55 (m, 6H, H-2', H-2''', H-5''', H-3', H-5', H-2'), 3.54–3.30 (m, 4H, H-4', H-3''', H-5'', H-3''), 3.22–3.14 (m, 2H, H-4''', H-4''), 3.04–2.90 (m, 2H, H-3, H-19), 2.28–2.16 (m, 1H, H-13), 2.14–2.06 (m, 1H, H-16a), 1.63 (s, 3H, H-30), 1.13 (d,  $J = 6.3$  Hz, 3H, H-6'), 1.11 (d,  $J = 6.3$  Hz, 3H, H-6''), 1.07 (d,  $J = 6.2$  Hz, 3H, H-6'''), 0.92 (s, 3H, H-27), 0.85 (s, 3H, H-23), 0.85 (s, 3H, H-26), 0.77 (s, 4H, H-25, H-5). <sup>13</sup>C NMR [400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO + 1 drop H<sub>2</sub>O]  $\delta$  185.5 (C-28), 150.4 (C-20), 109.7 (C-29), 102.9 (C-1'''), 102.6 (C-1'), 101.8 (C-1''), 88.0 (C-3), 79.6 (C-3'), 77.4 (C-4'), 71.9 (C-4'''), 71.7 (C-4''), 70.7 (C-2''), 70.7 (C-3''), 70.5 (C-3'''), 70.4 (C-2'), 70.4 (C-2'''), 69.3 (C-5''), 68.6 (C-5'''), 67.4 (C-5'), 55.5 (C-17), 54.7 (C-5), 49.9 (C-9), 48.6 (C-18), 46.7 (C-19), 42.1 (C-14), 40.3 (C-8), 38.4 (C-4), 38.0 (C-1), 37.6 (C-13), 36.6 (C-10), 36.5 (C-22), 33.9 (C-7), 31.8 (C-16), 30.2 (C-21), 29.3 (C-15), 27.8 (C-23), 25.2 (C-2), 25.2 (C-12),

20.5 (C-11), 19.0 (C-30), 18.1 (C-6'), 17.9 (C-6), 17.7 (C-6''), 17.7 (C-6'''), 16.2 (C-24), 15.9 (C-25), 15.8 (C-26), 14.4 (C-27). HRMS calcd for C<sub>48</sub>H<sub>78</sub>O<sub>15</sub> [M + Na]<sup>+</sup> 917.5232, found 917.5229.

**3-O- $\alpha$ -L-Rhamnopyranosyl-(1  $\rightarrow$  2)-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  3)]-[ $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  4)]- $\alpha$ -L-rhamnopyranosyl betulinic acid (6).** Based on the general procedure for inverse Schmidt glycosylation, compound **14** (70 mg, 103  $\mu$ mol) and TMSOTf (2  $\mu$ L, 10  $\mu$ mol) were dissolved in dry DCM (4 mL) with 4 Å molecular sieves (50 mg) at –78 °C. After stirring the solution during 30 min, a solution of 2,3,4-tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate **12** (320 mg, 515  $\mu$ mol) in dry DCM (2 mL) was added. The reaction mixture was stirred at –15 °C for 25 min, then the reaction was quenched with Et<sub>3</sub>N (0.2 equiv.) and filtered. The filtrate was concentrated under reduced pressure, and the residue was mixed with 10% Pd/C (2 mg) in dry EtOAc (5 mL). The reaction was refluxed under H<sub>2</sub> atmosphere. When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite® and concentrated under reduced pressure. The residue was suspended in dry MeOH–DCM (2 : 1, 3 mL) to which a freshly prepared solution of NaOMe (0.5 M, 3 mL) was added. After overnight stirring, reaction was neutralized to pH 7 with Dowex G-26 (H<sup>+</sup> form) and filtered. The filtrate was concentrated to dryness and purified by normal phase flash chromatography (26 : 14 : 3 CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O) and by preparative reversed-phase HPLC (Phenomenex Kinetex XB C<sub>18</sub> column, gradient elution with H<sub>2</sub>O : CH<sub>3</sub>CN 10  $\rightarrow$  100% at a flow rate of 20 mL min<sup>–1</sup> for 30 min, retention time: 18.944 min) to give **6** (40 mg, 37%, three steps)  $R_f = 0.19$  (26 : 14 : 3, CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –37.7 (c 0.1, CHCl<sub>3</sub>–MeOH 1 : 1). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub> 1 : 1)  $\delta$  4.97 (s, 1H, H-1''), 4.93 (s, 1H, H-1'''), 4.86 (s, 1H, H-1'''), 4.82 (s, 1H, H-1'), 4.73 (br s, 1H, H-29), 4.60 (m, 1H, H-29), 3.98 (dd,  $J = 9.7, 2.8$  Hz, 1H, H-3'), 3.93 (br s, 1H, H-2'''), 3.90 (br s, 1H, H-2'''), 3.88 (br s, 1H, H-2'), 3.82 (br s, 1H, H-2''), 3.80–3.55 (m, 8H, H-5', H-5'', H-3''', H-5''', H-5''', H-3'', H-3'', H-4'), 3.47–3.37 (m, 3H, H-4''', H-4'', H-4'''), 3.10–2.98 (m, 2H, H-3, H-19), 2.32–2.19 (m, 2H, H-13, H-16a), 2.02–1.88 (m, 2H, H-21a, H-22a), 1.69 (s, 3H, H-30), 1.33–1.23 (m, 12H, H-6'', H-6'', H-6''', H-6'), 0.99 (s, 3H, H-27), 0.95 (s, 3H, H-26), 0.92 (s, 3H, H-23), 0.85 (s, 3H, H-25), 0.77 (s, 3H, H-24), 0.72 (br d,  $J = 9.7$  Hz, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub> 1 : 1)  $\delta$  179.7 (C-28), 151.1 (C-20), 109.7 (C-29), 103.2 (C-1'''), 102.7 (C-1''), 102.6 (C-1'''), 101.7 (C-1'), 89.8 (C-3), 80.1 (C-4'), 79.8 (C-3'), 78.8 (C-2'), 73.0 (C-4'''), 72.9 (C-4''), 72.6 (C-4''), 71.6 (C-3''), 71.5 (C-3'''), 71.4 (C-3'''), 71.3 (C-2''), 71.0 (2 $\times$ , C-2''', C-2''), 69.7 (C-5''), 69.6 (C-5'''), 69.3 (C-5'''), 68.1 (C-5'), 56.6 (C-17), 55.8 (C-5), 50.9 (C-9), 49.6 (C-18), 47.4 (C-19), 42.8 (C-14), 41.1 (C-8), 39.5 (C-4), 39.0 (C-1), 38.7 (C-13), 37.5 (C-22), 37.3 (C-10), 34.7 (C-7), 32.7 (C-16), 31.0 (C-21), 30.0 (C-15), 28.4 (C-23), 26.0 (C-2), 25.9 (C-12), 21.3 (C-11), 19.5 (C-30), 18.6 (C-6), 18.2 (C-6'), 17.6 (C-6'''), 17.6 (C-6''), 17.3 (C-6''), 16.5 (C-24), 16.4 (C-25), 16.2 (C-26), 14.9 (C-27). HRMS calcd for C<sub>54</sub>H<sub>92</sub>O<sub>19</sub>N [M + NH<sub>4</sub>]<sup>+</sup> 1058.62581, found 1058.62553.

**3-O-(2,3,4-Tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-28-benzyl ursolate (21).** Based on the general procedure for normal Schmidt glycosylation, a solution of benzyl ursolate **20** (ref. 37) (270 mg, 507  $\mu$ mol) and **12** (377 mg, 608  $\mu$ mol) in DCM (8 mL)



were added TMSOTf (9.2  $\mu$ L, 51  $\mu$ mol) and stirred for 30 min. After filtration and concentration to dryness, the residue was purified by flash chromatography (8 : 2, hexanes–EtOAc) to yield **21** as a white powder (342 mg, 68%);  $R_f = 0.52$  (8 : 2, hexanes–EtOAc);  $[\alpha]_D^{20} +82.6$  (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (d,  $J = 7.2$  Hz, 2H, CH–Bz), 7.99 (d,  $J = 7.2$  Hz, 2H, CH–Bz), 7.84 (d,  $J = 7.2$  Hz, 2H, CH–Bz), 7.60 (t,  $J = 7.4$  Hz, 2H, CH–Bz), 7.55–7.22 (m, 13H, CH–Bz, CH–Bn), 5.84 (dd,  $J = 10.2, 3.3$  Hz, 1H, H-3'), 5.69 (t,  $J = 10.0$  Hz, 1H, H-4'), 5.65 (dd,  $J = 3.2, 1.7$  Hz, 1H, H-2'), 5.25 (t,  $J = 3.1$  Hz, 1H, H-12), 5.12 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>–Bn), 5.09 (br s, 1H, H-1'), 4.99 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>–Bn), 4.32 (dq,  $J = 9.7, 6.1$  Hz, 1H, H-5'), 3.24 (m, 1H, H-3), 2.27 (d,  $J = 11.3$  Hz, 1H, H-18) 1.34 (d,  $J = 6.3$  Hz, 3H, H-6'), 1.08 (s, 3H, H-27), 1.07 (s, 3H, H-23), 0.97 (s, 3H, H-25), 0.96 (s, 3H, H-24), 0.93 (d,  $J = 6.1$  Hz, 3H, H-30), 0.86 (d,  $J = 6.4$  Hz, 3H, H-29), 0.76 (d,  $J = 11.2$  Hz, 1H, H-5), 0.66 (s, 3H, H-26). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  177.3 (C-28), [165.8, 165.7, 165.6 (CO–Bz)], 138.1 (C-13), 136.4 (C-Bn), [133.4, 133.3, 133.1, 129.9, 129.7<sub>3</sub>, 129.6<sub>6</sub> (CH–Bz)], [129.5, 129.3<sub>2</sub>, 129.2<sub>5</sub> (C–Bz)], [128.6, 128.4 (2 $\times$ ), 128.3, 128.1, 127.9 (CH–Bz, CH–Bn)], 125.6 (C-12), 99.7 (C-1'), 90.0 (C-3), 72.0 (C-4'), 71.2 (C-2'), 70.2 (C-3'), 66.7 (C-5'), 66.0 (CH<sub>2</sub>–Bn), 55.4 (C-5), 52.9 (C-18), 48.1 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.6 (C-1), 36.7 (C-10), 36.6 (C-16), 33.0 (C-7), 30.6 (C-21), 28.4 (C-23), 27.9 (C-15), 25.5 (C-2), 24.2 (C-22), 23.5 (C-27), 23.3 (C-11), 21.2 (C-30), 18.3 (C-6), 17.6 (C-6'), 17.0<sub>1</sub> (C-26), 17.9<sub>8</sub> (C-29), 16.6 (C-24), 15.5 (C-25). HRMS calcd for C<sub>64</sub>H<sub>76</sub>O<sub>16</sub>Na [M + Na]<sup>+</sup> 1027.53307, found 1027.53329.

**3-O- $\alpha$ -l-Rhamnopyranosyl-28-benzyl ursolate (22).** Based on the general procedure for benzoyl groups removal, compound **21** (300 mg, 303  $\mu$ mol) was dissolved in MeOH–DCM (2 : 1, 5 mL) with NaOMe in MeOH (0.5 M, 5 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (9 : 1, DCM–MeOH) to give compound **22** (202 mg, 98%) as amorphous solid,  $R_f = 0.21$  (7 : 1, CHCl<sub>3</sub> : MeOH),  $[\alpha]_D^{20} +1.3$  (c 7, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.36–7.31 (m, 5H, CH–Bn), 5.23 (t,  $J = 3.1$  Hz, 1H, H-12), 5.11 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>–Bn), 4.97 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>–Bn), 4.80 (br s, 1H, H-1'), 3.93 (br s, 1H, H-2'), 3.79–3.74 (m, 2H, H-3', H-5'), 3.47 (m, 1H, H-4'), 3.07 (dd,  $J = 10.5, 4.4$  Hz, 1H, H-3), 2.26 (d,  $J = 11.1$  Hz, 1H, H-18), 1.27 (d,  $J = 6.0$  Hz, 1H, H-6'), 1.06 (s, 3H, H-27), 0.93 (s, 3H, H-30), 0.90 (s, 3H, H-23), 0.89 (s, 3H, H-25) 0.85 (d,  $J = 6.2$  Hz, 3H, H-29), 0.75 (s, 3H, H-24), 0.69 (d,  $J = 11.1$  Hz, 1H, H-5), 0.63 (s, 3H, H-26). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  177.3 (C-28), 138.1 (C-13), 136.4 (C-Bn), [128.4 (2 $\times$ ), 128.1 (2 $\times$ ), 127.9 (CH–Bn)], 125.7 (C-12), 102.2 (C-1'), 89.6 (C-3), 73.8 (C-4'), 72.0 (C-3'), 71.3 (C-2'), 67.7 (C-5'), 66.0 (CH<sub>2</sub>–Bn), 55.4 (C-5), 52.9 (C-18), 48.1 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.6 (C-1), 36.6 (C-22), 36.6 (C-10), 33.0 (C-7), 30.6 (C-21), 28.3 (C-23), 27.9 (C-15), 25.3 (C-2), 24.2 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.2 (C-6), 17.4 (C-6'), 17.0<sub>4</sub> (C-29), 16.9<sub>8</sub> (C-26), 16.5 (C-24), 15.5 (C-25). HRMS calcd for C<sub>43</sub>H<sub>64</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup> 715.45443, found 715.45649.

**3-O- $\alpha$ -l-Rhamnopyranosyl ursolic acid (7).** In parallel, UA 3-O- $\alpha$ -l-rhamnopyranoside **7** was synthesized in two steps starting from **21**. Therefore, a solution of compound **21** (54 mg, 54  $\mu$ mol) and 10% Pd/C (1 mg) in dry EtOAc (3 mL) was refluxed and

purged with H<sub>2</sub> for 2 h. When TLC indicated the disappearance of the starting material, the residue was filtered over Celite® and concentrated before engaged on the benzoyl groups removal as described on general procedure 5.1.3 with NaOMe in MeOH (0.5 M, 2 mL) in a mixture of MeOH–DCM (2 : 1, 2 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (7 : 1, DCM–MeOH) to give compound **7** (24 mg, 74%, two steps) as colorless oil,  $R_f = 0.3$  (DCM: MeOH, 7 : 1),  $[\alpha]_D^{20} +5.7$  (c 0.74, CHCl<sub>3</sub>–MeOH 1 : 1). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub> 1 : 1)  $\delta$  5.24 (br s, 1H, H-12), 4.77 (br s, 1H, H-1'), 3.89 (dd,  $J = 3.4, 1.7$  Hz, 1H, H-2'), 3.76 (dq,  $J = 9.5, 6.2$  Hz, 1H, H-5'), 3.70 (dd,  $J = 9.5, 3.3$  Hz, 1H, H-3'), 3.39 (t,  $J = 9.5$  Hz, 1H, H-4'), 3.10 (dd,  $J = 11.2, 4.7$  Hz, 1H, H-3), 2.20 (d,  $J = 11.3$  Hz, 1H, H-18), 1.27 (d,  $J = 6.1$  Hz, 3H, H-6'), 1.10 (s, 3H, H-27), 0.96 (m, 3H, H-30), 0.95 (s, 3H, H-23), 0.95 (s, 3H, H-25), 0.88 (d,  $J = 6.4$  Hz, 3H, H-29), 0.83 (s, 3H, H-26), 0.78 (s, 3H, H-24). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub> 1 : 1)  $\delta$  181.1 (C-28), 138.7 (C-13), 125.9 (C-12), 103.2 (C-1'), 89.7 (C-3), 73.4 (C-4'), 71.9 (C-3'), 71.5 (C-2'), 68.7 (C-5'), 55.8 (C-5), 53.3 (C-18), 48.2 (C-17), 48.0 (C-9), 42.5 (C-14), 39.9 (C-8), 39.6 (C-19), 39.4 (C-20), 39.3 (C-4), 39.0 (C-1), 37.3 (C-22), 37.1 (C-10), 33.5 (C-7), 31.1 (C-21), 30.0 (C-), 28.5 (C-23), 28.5 (C-15), 25.8 (C-2), 24.6 (C-16), 23.8 (C-27), 23.7 (C-11), 21.4 (C-30), 18.7 (C-6), 17.5 (C-6'), 17.3 (C-29), 17.2 (C-26), 16.7 (C-24), 15.7 (C-25). HRMS calcd for C<sub>36</sub>H<sub>58</sub>O<sub>7</sub> [M + H]<sup>+</sup> 603.4255, found 603.4273.

**3-O-(2,3-Di-O-isopropylidene- $\alpha$ -l-rhamnopyranosyl)-28-benzyl ursolate (23).** Compound **22** (500 mg, 736  $\mu$ mol) was dissolved in acetone (10 mL) to which was added 2,2-dimethoxypropane (272  $\mu$ L, 2209  $\mu$ mol) and PTSA (12.7 mg, 74  $\mu$ mol) as catalyst. After 2 h, reaction was evaporated to dryness and purified by flash chromatography (7 : 3, hexanes–EtOAc) to give compound **23** (392 mg, 74%) as colorless oil,  $R_f = 0.7$  (hexanes–EtOAc, 7 : 3),  $[\alpha]_D^{20} +6.7$  (c 10, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.38–7.28 (m, 5H, CH–Bn), 5.23 (t,  $J = 3.1$  Hz, 1H, H-12), 5.10 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>–Bn), 4.99 (s, 1H, H-1'), 4.97 (d,  $J = 12.2$  Hz, 1H, CH<sub>2</sub>–Bn), 3.16–4.10 (m, 2H, H-2', H-3'), 3.82 (dq,  $J = 12.6, 6.2$  Hz, 1H, H-5'), 3.42 (t, 1H,  $J = 8.5$  Hz, H-4'), 3.15 (dd,  $J = 10.8, 4.3$  Hz, 1H, H-3), 2.26 (d,  $J = 11.1$  Hz, 1H, H-18), 1.99 (dd,  $J = 12.8, 4.1$  Hz, 1H), 1.53 (s, 3H, CH<sub>3</sub>–iso), 1.37 (s, 3H, CH<sub>3</sub>–iso), 1.26 (d,  $J = 6.6$  Hz, 3H, H-6'), 1.07 (s, 3H, H-27), 0.94 (s, 6H, H-23, H-30), 0.90 (s, 3H, H-25), 0.85 (d,  $J = 6.3$  Hz, 3H, H-29), 0.77 (s, 3H, H-24), 0.72 (d, 1H,  $J = 11.3$  Hz H-5), 0.63 (s, 3H, H-26). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  177.3 (C-28), 138.0 (C-13), 136.3 (C-Bn), [128.4 (2 $\times$ ), 128.1 (2 $\times$ ), 127.9 (CH–Bn)], 125.7 (C-12), 109.3 (C-iso), 99.7 (C-1'), 88.9 (C-3), 78.3 (C-3'), 75.8 (C-2'), 74.1 (C-4'), 66.2 (C-5'), 66.0 (CH<sub>2</sub>–Bn), 55.3 (C-5), 52.8 (C-18), 48.1 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.6 (C-1), 36.6 (C-10), 36.6 (C-22), 32.9 (C-7), 30.6 (C-21), 28.4 (C-23), 27.9 (CH<sub>3</sub>–iso), 27.9 (C-15), 26.2 (CH<sub>3</sub>–iso), 25.4 (C-2), 24.2 (C-16), 23.5 (C-27), 23.2 (C-11), 21.2 (C-30), 18.2 (C-6), 17.5 (C-6'), 17.0 (C-29), 17.0 (C-26), 16.5 (C-24), 15.5 (C-25). HRMS calcd for C<sub>46</sub>H<sub>68</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup> 755.48573, found 755.48487.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -l-rhamnopyranosyl)-(1 $\rightarrow$ 4)-2,3-di-O-isopropylidene- $\alpha$ -l-rhamnopyranosyl)-28-benzyl ursolate (24).** Based on the general procedure for normal Schmidt glycosylation, compound **23** (191 mg, 266  $\mu$ mol) and 2,3,4-tri-O-benzoyl-



$\alpha$ -L-rhamnopyranosyl trichloroacetimidate **12** (198 mg, 319  $\mu$ mol) was coupled in DCM (8 mL) with TMSOTf (4.8  $\mu$ L, 26  $\mu$ mol) as Lewis acid for 25 min. When TLC indicated completion, reaction was then quenched with Et<sub>3</sub>N (12  $\mu$ L) filtered and evaporated before purification by flash chromatography (8 : 2, hexanes–EtOAc) to give compound **24** (219 mg, 70%) as colorless oil,  $R_f$  = 0.6 (hexanes–EtOAc, 8 : 2),  $[\alpha]_D^{20}$  +36.1 (c 2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.12 (d,  $J$  = 7.2 Hz, 2H, CH-Bz), 7.96 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.81 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.61 (t,  $J$  = 7.5 Hz, 1H, CH-Bz), 7.54–7.46 (m, 3H, CH-Bz), 7.44–7.30 (m, 7H, CH-Bz, CH-Bn), 7.28–7.22 (m, 3H, CH-Bz), 5.77 (dd,  $J$  = 10.1, 3.3 Hz, 1H, H-3''), 5.74 (dd,  $J$  = 3.1, 1.8 Hz, 1H, H-2''), 5.67 (t,  $J$  = 9.8 Hz, 1H, H-4''), 5.59 (d,  $J$  = 1.7 Hz, 1H, H-1''), 5.26 (br s, 1H, H-12), 5.12 (d,  $J$  = 12.5 Hz, 1H, CH<sub>2</sub>-Bn), 5.04 (br s, 1H, H-1'), 4.99 (d,  $J$  = 12.5 Hz, 1H, CH<sub>2</sub>-Bn), 4.31 (dd,  $J$  = 7.2, 5.5 Hz, 1H, H-3'), 4.20 (dq,  $J$  = 9.6, 6.4 Hz, 1H, H-5''), 4.14 (d,  $J$  = 5.5 Hz, 1H, H-2'), 3.98 (dq,  $J$  = 10.0, 6.1 Hz, 1H, H-5'), 3.62 (dd,  $J$  = 9.9, 7.3 Hz, 1H, H-4'), 3.17 (dd,  $J$  = 9.2, 6.9 Hz, 1H, H-3), 2.28 (d,  $J$  = 11.3 Hz, 1H, H-18), 2.02 (td,  $J$  = 12.7, 3.9 Hz, 1H, H-16), 1.55 (s, 3H, CH<sub>3</sub>-iso), 1.38 (m, 6H, H-6', H-6''), 1.35 (s, 3H, CH<sub>3</sub>-iso), 1.08 (s, 3H, H-27), 0.96 (s, 3H, H-23), 0.94 (m, 6H, H-30, H-25), 0.86 (d,  $J$  = 6.2 Hz, H-29), 0.83 (s, 3H, H-24), 0.75 (br d,  $J$  = 11.5 Hz, 1H, H-5), 0.66 (s, 3H, H-26). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  177.3 (C-28), [165.7, 165.6, 165.4 (CO-Bz)], 138.1 (C-13), 136.4 (C-Bn), [133.4, 133.3, 133.1, 130.0 (2 $\times$ ), 129.7<sub>1</sub> (2 $\times$ ), 129.6<sub>8</sub> (2 $\times$ ) (CH-Bz)], [129.5, 129.3, 129.2 (C-Bz)], [128.5 (2 $\times$ ), 128.4 (4 $\times$ ), 128.2<sub>5</sub> (2 $\times$ ), 128.1<sub>6</sub> (2 $\times$ ), 127.9 (CH-Bz, CH-Bn)], 125.7 (C-12), 109.5 (C-iso), 99.5 (C-1'), 96.0 (C-1''), 89.0 (C-3), 78.2 (C-3'), 77.9 (C-4'), 76.3 (C-2'), 71.7 (C-4''), 70.8 (C-2''), 70.0 (C-3''), 67.3 (C-5''), 66.0 (C-7), 63.7 (C-5'), 55.4 (C-5), 52.9 (C-18), 48.1 (C-17), 47.6 (C-9), 42.0 (C-14), 39.5 (C-8), 39.0<sub>9</sub> (C-19), 39.0<sub>8</sub> (C-4), 38.8 (C-20), 38.7 (C-1), 36.7 (2 $\times$ , C-22, C-10), 33.0 (C-7), 30.7 (C-21), 28.4 (C-23), 28.0 (CH<sub>3</sub>-iso), 27.9 (C-15), 26.5 (CH<sub>3</sub>-iso), 25.5 (C-2), 24.2 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.3 (C-6), 18.1 (C-6'), 17.7 (C-6''), 17.0 (C-29), 17.0 (C-26), 16.6 (C-24), 15.5 (C-25). HRMS calcd for C<sub>73</sub>H<sub>90</sub>O<sub>18</sub>N [M + NH<sub>4</sub>]<sup>+</sup> 1208.6668, found 1208.6647.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl)-28-benzyl ursolate (25).** To a solution of compound **24** (161 mg, 137  $\mu$ mol) in DCM (5 mL) was added HOAc 80% aq. (15 mL). After 3 h stirring at 80 °C, the reaction was evaporated to dryness and the crude was purified by flash chromatography (8 : 2, hexanes–EtOAc) to give compound **25** (129.1 mg, 83%) as a colorless oil,  $R_f$  = 0.5 (hexanes–EtOAc, 8 : 2),  $[\alpha]_D^{20}$  +37.7 (c 4.1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.07 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.97 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.82 (d,  $J$  = 7.3 Hz, 2H, CH-Bz), 7.58 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.51 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.48–7.30 (m, 10H, CH-Bz), 7.28–7.22 (m, 2H, CH-Bz), 5.82 (dd,  $J$  = 10.1, 3.2 Hz, 1H, H-3''), 5.79 (m, 1H, H-2''), 5.68 (t,  $J$  = 9.9 Hz, 1H, H-4''), 5.49 (br s, 1H, H-1''), 5.25 (br s, 1H, H-12), 5.11 (d,  $J$  = 12.5 Hz, 1H, CH<sub>2</sub>-Bn), 4.99 (d,  $J$  = 12.5 Hz, 1H, CH<sub>2</sub>-Bn), 4.86 (br s, 1H, H-1'), 4.30 (dq,  $J$  = 9.6, 6.2 Hz, 1H, H-5''), 4.07 (dd,  $J$  = 9.0, 2.8 Hz, 1H, H-3'), 4.01–3.92 (m, 2H, H-2', H-5'), 3.64 (m, 1H, H-4'), 3.11 (dd,  $J$  = 10.5, 5.4 Hz, 1H, H-3), 2.27 (d,  $J$  = 11.1 Hz, 1H, H-18), 1.37 (m, 6H, H-6', H-6''), 1.08 (s, 3H, H-27), 0.94 (m, 3H, H-30), 0.93 (s, 3H, H-25), 0.91 (s, 3H, H-23), 0.86 (d,  $J$  = 6.4 Hz, 3H, H-29), 0.81 (s, 3H, H-24), 0.71 (br d,  $J$  = 11.4 Hz, 1H, H-5), 0.65 (s, 3H, H-26). <sup>13</sup>C NMR (101

MHz, CDCl<sub>3</sub>)  $\delta$  177.4 (C-28), [165.9, 165.8, 165.7 (CO-Bz)], 138.1 (C-13), 136.3 (C-Bn), [133.5, 133.3, 133.2, 129.9 (2 $\times$ ), 129.7 (4 $\times$ ) (CH-Bz)], [129.3, 129.2, 129.1 (C-Bz)], [128.5 (2 $\times$ ), 128.4 (4 $\times$ ), 128.3 (2 $\times$ ), 128.1 (2 $\times$ ), 127.9 (CH-Bz, CH-Bn)], 125.7 (C-12), 102.0 (C-1'), 99.0 (C-1''), 89.5 (C-3), 81.6 (C-4'), 71.9 (C-2'), 71.9 (C-3'), 71.6 (C-4''), 71.2 (C-2''), 70.1 (C-3''), 67.5 (C-5''), 66.3 (C-5'), 66.0 (CH<sub>2</sub>-Bn), 55.3 (C-5), 52.9 (C-18), 48.1 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 38.9 (C-4), 38.8 (C-20), 38.6 (C-1), 36.6 (C-22), 36.6 (C-10), 33.0 (C-7), 30.6 (C-21), 28.3 (C-23), 27.9 (C-15), 25.4 (C-2), 24.2 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.2 (C-6), 17.9 (C-6'), 17.6 (C-6''), 17.0 (C-29), 17.0 (C-26), 16.6 (C-24), 15.4 (C-25). HRMS calcd for C<sub>70</sub>H<sub>90</sub>O<sub>14</sub>N [M + NH<sub>4</sub>]<sup>+</sup> 1168.6355, found 1168.6351.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl)ursolic acid (26).** A solution of compound **25** (40 mg, 35  $\mu$ mol) and 10% Pd/C (2 mg) in dry EtOAc (3 mL) was refluxed and purged with H<sub>2</sub>. When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite® and concentrated under reduced pressure. The residue was purified by flash chromatography (7 : 3, hexanes–EtOAc) to give compound **26** (33 mg, 89%) as a colorless oil,  $R_f$  = 0.6 (hexanes–EtOAc, 7 : 3),  $[\alpha]_D^{20}$  +31.3 (c 3, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.05 (d,  $J$  = 7.7 Hz, 2H, CH-Bz), 7.97 (d,  $J$  = 7.8 Hz, 2H, CH-Bz), 7.82 (d,  $J$  = 7.8 Hz, 2H, CH-Bz), 7.58 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.52 (t,  $J$  = 7.4 Hz, 1H, CH-Bz), 7.45 (t,  $J$  = 7.6 Hz, 2H, CH-Bz), 7.43–7.36 (m, 3H, CH-Bz), 7.28–7.23 (m, 2H, CH-Bz), 5.81 (dd,  $J$  = 10.2, 3.4 Hz, 1H, H-3''), 5.75 (br s, 1H, H-2''), 5.68 (t,  $J$  = 9.9 Hz, 1H, H-4''), 5.50 (s, 1H, H-1''), 5.26 (br s, 1H, H-12), 4.86 (s, 1H, H-1'), 4.29 (dq,  $J$  = 9.6, 6.5 Hz, 1H, H-5''), 4.10 (dd,  $J$  = 9.1, 3.3 Hz, 1H, H-3'), 4.01–3.93 (m, 2H, H-2', H-5'), 3.64 (t,  $J$  = 9.2 Hz, 1H, H-4'), 3.13 (dd,  $J$  = 9.5, 6.0 Hz, 1H, H-3), 2.19 (br d,  $J$  = 11.1 Hz, 1H, H-18), 1.38 (d,  $J$  = 6.0 Hz, 3H, H-6'), 1.36 (d,  $J$  = 6.5 Hz, 3H, H-6''), 1.08 (s, 3H, H-27), 0.98 (s, 3H, H-25), 0.95 (s, 3H, H-23), 0.95 (m, 3H, H-30), 0.87 (d,  $J$  = 6.3 Hz, 3H, H-29), 0.84 (s, 3H, H-24), 0.79 (s, 3H, H-26), 0.75 (m, 1H, H-5). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  182.9 (C-28), [165.9 (2 $\times$ ), 165.8 (CO-Bz)], 137.9 (C-13), [133.5, 133.4, 133.2, 130.0 (2 $\times$ ), 129.7 (4 $\times$ ) (CH-Bz)], [129.3 (2 $\times$ ), 129.1 (C-Bz)], [128.6 (2 $\times$ ), 128.4 (2 $\times$ ), 128.3 (2 $\times$ ) (CH-Bz)], 125.9 (C-12), 101.9 (C-1'), 98.8 (C-1''), 89.6 (C-3), 81.2 (C-4'), 72.0 (C-2'), 71.9 (C-3'), 71.6 (C-4''), 71.2 (C-2''), 70.0 (C-3''), 67.5 (C-5''), 66.2 (C-5'), 55.4 (C-5), 52.6 (C-18), 47.9 (C-17), 47.5 (C-9), 41.9 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.6 (C-1), 36.7 (2 $\times$ , C-22, C-10), 32.9 (C-7), 30.6 (C-21), 28.4 (C-23), 28.0 (C-15), 25.5 (C-2), 24.1 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.3 (C-6), 18.0 (C-6'), 17.6 (C-6''), 17.1 (C-26), 17.0 (C-29), 16.6 (C-24), 15.5 (C-25). HRMS calcd for C<sub>63</sub>H<sub>80</sub>O<sub>14</sub>Na [M + Na]<sup>+</sup> 1083.54403, found 1083.54598.

**3-O- $\alpha$ -L-Rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl ursolic acid (8).** Based on the general procedure for benzoyl groups removal, compound **26** (33 mg, 32  $\mu$ mol) was dissolved in MeOH–DCM (2 : 1, 3 mL) with NaOMe in MeOH (0.5 M, 3 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 (H<sup>+</sup> form), filtered and purified by flash chromatography (9 : 1 DCM–MeOH  $\rightarrow$  26 : 14 : 3 DCM–MeOH–H<sub>2</sub>O) to give compound **8** (20 mg, 86%) as a colorless oil,  $R_f$  = 0.2 (DCM: MeOH, 9 : 1),  $[\alpha]_D^{20}$  –40.0 (c 0.5, 1 : 1, CHCl<sub>3</sub>–MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD 1 : 1)  $\delta$  5.24



(m, 1H, H-12), 5.20 (d,  $J = 1.3$  Hz, 1H, H-1''), 4.75 (m, 1H, H-1'), 3.98 (dd,  $J = 3.2, 1.8$  Hz, 1H, H-2''), 3.82 (dd,  $J = 3.1, 1.7$  Hz, 1H, H-2'), 3.79 (dd,  $J = 9.2, 3.3$  Hz, 1H, H-3'), 3.76–3.70 (m, 2H, H-5', H-5''), 3.68 (dd,  $J = 9.5, 3.3$  Hz, 1H, H-3''), 3.52 (t,  $J = 9.3$  Hz, 1H, H-4'), 3.41 (t,  $J = 9.5$  Hz, 1H, H-4''), 3.11 (dd,  $J = 10.7, 4.7$  Hz, 1H, H-3), 2.20 (br d,  $J = 11.4$  Hz, 1H, H-18), 2.02 (td,  $J = 13.3, 3.6$  Hz, 1H, H-16a), 1.28 (d,  $J = 6.1$  Hz, 6H, H-6', H-6''), 1.11 (s, 3H, H-27), 0.96 (m, 9H, H-25, H-30, H-23), 0.88 (d,  $J = 6.4$  Hz, 3H, H-29), 0.84 (s, 3H, H-26), 0.80 (s, 3H, H-24).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3/\text{CD}_3\text{OD}$  1 : 1)  $\delta$  180.3 (C-28), 138.9 (C-13), 126.1 (C-12), 103.3 (C-1'), 102.3 (C-1''), 89.9 (C-3), 80.4 (C-4'), 73.2 (C-4''), 72.7 (C-3'), 72.2 (C-2'), 71.7 (C-3''), 71.5 (C-2''), 69.6 (C-5''), 67.5 (C-5'), 56.0 (C-5), 53.5 (C-18), 48.2 (C-9), 48.0 (C-17), 42.6 (C-14), 40.1 (C-8), 39.7 (C-19), 39.6 (C-20), 39.5 (C-4), 39.2 (C-22), 37.5 (C-1), 37.2 (C-10), 33.6 (C-7), 31.2 (C-21), 28.6 (C-15), 28.5 (C-23), 26.0 (C-2), 24.8 (C-16), 23.9 (C-27), 23.8 (C-11), 21.4 (C-30), 18.8 (C-6), 18.2 (C-6'), 17.6 (C-6''), 17.4 (C-26), 17.3 (C-29), 16.8 (C-24), 15.8 (C-25). HRMS calcd for  $\text{C}_{42}\text{H}_{68}\text{O}_{11}\text{Na}$  [ $\text{M} + \text{Na}$ ] $^+$  771.4653, found 771.4657.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl]-28-benzyl ursolate (27).** Based on the general procedure for normal Schmidt glycosylation, compound 25 (94 mg, 83  $\mu\text{mol}$ ) and 2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate 12 (56 mg, 91  $\mu\text{mol}$ ) was coupled in DCM (5 mL) with TMSOTf (1.5  $\mu\text{L}$ , 8.3  $\mu\text{mol}$ ) as Lewis acid for 10 min. When TLC indicated completion, reaction was then quenched with  $\text{Et}_3\text{N}$  (5  $\mu\text{L}$ ) filtered and evaporated before purification by flash chromatography (7 : 3, hexanes–EtOAc) to give compound 27 (83 mg, 62%) as colorless oil,  $R_f = 0.6$  (hexanes–EtOAc, 8 : 2),  $[\alpha]_{\text{D}}^{20} -1.0$  (c 4.4,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.02–7.92 (m, 8H, CH-Bz), 7.89 (d,  $J = 8.0$  Hz, 2H, CH-Bz), 7.77 (d,  $J = 8.0$  Hz, 2H, CH-Bz), 7.56–7.23 (m, 19H, CH-Bz), 7.17 (t,  $J = 7.8$  Hz, 2H, CH-Bz), 7.10 (t,  $J = 7.8$  Hz, 2H, CH-Bz), 6.04 (dd,  $J = 3.5, 1.8$  Hz, 1H, H-2'''), 5.98 (dd,  $J = 10.1, 3.3$  Hz, 1H, H-3'''), 5.95 (dd,  $J = 10.2, 3.1$  Hz, 1H, H-3''), 5.83 (dd,  $J = 3.4, 1.9$  Hz, 1H, H-2''), 5.75 (t,  $J = 10.0$  Hz, 1H, H-4''), 5.67 (t,  $J = 9.9$  Hz, 1H, H-4'''), 5.54 (br s, 1H, H-1''), 5.37 (br s, 1H, H-1'''), 5.25 (br s, 1H, H-12), 5.10 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>-Bn), 4.99 (d,  $J = 12.5$  Hz, 1H, CH<sub>2</sub>-Bn), 4.87 (br s, 1H, H-1'), 4.47 (dq,  $J = 9.8, 6.3$  Hz, 1H, H-5'''), 4.32 (dq,  $J = 9.8, 5.9$  Hz, 1H, H-5''), 4.22–4.16 (m, 2H, H-2', H-3'), 4.04 (dq,  $J = 9.7, 6.3$  Hz, 1H, H-5'), 3.85 (t,  $J = 9.3$  Hz, 1H, H-4'), 3.14 (t,  $J = 7.9$  Hz, 1H, H-3), 2.27 (d,  $J = 11.1$  Hz, 1H, H-18), 1.43 (d,  $J = 6.1$  Hz, 3H, H-6'), 1.36 (d,  $J = 6.8$  Hz, 3H, H-6''), 1.35 (d,  $J = 7.1$  Hz, 3H, H-6'''), 1.07 (s, 3H, H-27), 0.96 (s, 3H, H-25), 0.93 (m, 6H, H-23, H-30), 0.85 (m, 6H, H-24, H-29), 0.73 (br d,  $J = 12.0$  Hz, 1H, H-5), 0.65 (s, 3H, H-26).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  177.3 (C-28), [165.8<sub>7</sub>, 165.8<sub>5</sub>, 165.6, 165.5, 165.1, 164.9 (CO-Bz)], 138.1 (C-13), 136.4 (C-Bn), [133.2<sub>5</sub>, 133.2<sub>2</sub>, 133.1, 132.8<sub>4</sub>, 132.7<sub>6</sub>, 132.6, 129.9 (4 $\times$ ), 129.8<sub>1</sub> (2 $\times$ ), 129.7<sub>7</sub> (4 $\times$ ) (CH-Bz)], [129.7<sub>1</sub>, 129.6<sub>3</sub>, 129.5<sub>8</sub>, 129.5<sub>1</sub>, 129.4, 129.3 (C-Bz)], [128.4<sub>1</sub> (2 $\times$ ), 128.3<sub>9</sub> (2 $\times$ ), 128.3<sub>5</sub> (2 $\times$ ), 128.3<sub>3</sub> (2 $\times$ ), 128.2<sub>6</sub>, 128.2<sub>2</sub> (2 $\times$ ), 128.1<sub>7</sub> (2 $\times$ ), 128.1 (2 $\times$ ), 127.9<sub>7</sub> (2 $\times$ ), 127.9<sub>6</sub> (2 $\times$ ) (CH-Bz, CH-Bn)], 125.7 (C-12), 101.9 (C-1'), 99.7 (C-1''), 99.5 (C-1'''), 89.9 (C-3), 81.7 (C-3'), 81.0 (C-4'), 72.0 (C-4''), 72.0 (C-4'''), 72.0 (C-2''), 71.3 (C-2'''), 71.1 (C-2'), 69.5 (C-3'''), 69.5 (C-3''), 67.7 (C-5'''), 67.6 (C-5''), 67.1 (C-5'), 55.4 (C-5), 52.6 (C-18), 47.9 (C-17), 47.6 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.7 (C-1), 36.7 (C-10), 36.6 (C-22), 32.9 (C-7), 30.5 (C-21), 28.4 (C-23), 28.0 (C-15), 25.5 (C-2), 24.2 (C-16), 23.6 (C-27), 23.4 (C-11), 21.2 (C-30), 18.8 (C-6), 18.3 (C-6'), 17.7 (C-6''), 17.6 (C-6'''), 17.0 (C-29), 16.9 (C-26), 16.7 (C-24), 15.5 (C-25). HRMS calcd for  $\text{C}_{90}\text{H}_{106}\text{O}_{21}\text{N}$  [ $\text{M} + \text{NH}_4$ ] $^+$  1536.7252, found 1536.7236.

67.1 (C-5'), 66.0 (C-7), 55.4 (C-5), 52.9 (C-18), 48.1 (C-17), 47.6 (C-9), 42.1 (C-14), 39.6 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.7 (C-1), 36.7 (C-10), 36.7 (C-22), 33.0 (C-7), 30.7 (C-21), 28.4 (C-23), 28.0 (C-15), 25.7 (C-2), 24.3 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.3 (C-6'), 18.3 (C-6), 17.7 (C-6''), 17.6 (C-6'''), 17.0 (C-29), 17.0 (C-26), 16.7 (C-24), 15.5 (C-25). HRMS calcd for  $\text{C}_{97}\text{H}_{112}\text{O}_{11}\text{N}$  [ $\text{M} + \text{NH}_4$ ] $^+$  1626.7721, found 1626.7724.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl]ursolic acid (28).** A solution of compound 27 (44 mg, 27  $\mu\text{mol}$ ) and 10% Pd/C (2 mg) in dry EtOAc (3 mL) was refluxed and purged with  $\text{H}_2$ . When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite® and concentrated under reduced pressure. The residue was purified by flash chromatography (8 : 2, hexanes–EtOAc) to give compound 28 (33.2 mg, 81%) as a colorless oil,  $R_f = 0.3$  (hexanes–EtOAc, 8 : 2),  $[\alpha]_{\text{D}}^{20} +60.6$  (c 3.4,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.01–7.92 (m, 8H, CH-Bz), 7.88 (d,  $J = 7.5$  Hz, 2H, CH-Bz), 7.77 (d,  $J = 7.6$  Hz, 2H, CH-Bz), 7.54–7.25 (m, 9H, CH-Bz), 7.16 (t,  $J = 7.7$  Hz, 2H, CH-Bz), 7.10 (t,  $J = 7.8$  Hz, 2H, CH-Bz), 6.04 (br s, 1H, H-2'''), 6.00 (dd,  $J = 10.0, 3.0$  Hz, 1H, H-3'''), 5.95 (dd,  $J = 10.0, 3.0$  Hz, 1H, H-3''), 5.83 (br s, 1H, H-2''), 5.76 (t,  $J = 9.9$  Hz, 1H, H-4''), 5.67 (t,  $J = 10.0$  Hz, 1H, H-4'''), 5.54 (s, 1H, H-1''), 5.38 (s, 1H, H-1'''), 5.26 (br s, 1H, H-12), 4.88 (s, 1H, H-1'), 4.47 (dq,  $J = 10.0, 6.4$  Hz, 1H, H-5'''), 4.32 (dq,  $J = 9.7, 6.4$  Hz, 1H, H-5''), 4.22–4.15 (m, 2H, H-2', H-3'), 4.04 (m, H-5'), 3.85 (t,  $J = 8.7$  Hz, 1H, H-4'), 3.15 (t,  $J = 8.0$  Hz, 1H, H-3), 2.19 (br d,  $J = 10.9$  Hz, 1H, H-18), 1.43 (d,  $J = 6.1$  Hz, 3H, H-6'), 1.37 (d,  $J = 6.1$  Hz, 3H, H-6''), 1.35 (d,  $J = 6.0$  Hz, 3H, H-6'''), 1.09 (s, 3H, H-27), 0.99 (s, 3H, H-25), 0.96 (m, 6H, H-30, H-23), 0.86 (m, 6H, H-29, H-24), 0.80 (s, 3H, H-26), 0.75 (br d,  $J = 11.7$  Hz, 1H, H-5).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  182.5 (C-28), [165.8<sub>6</sub>, 165.8<sub>5</sub>, 165.6, 165.5, 165.1, 164.9 (CO-Bz)], 137.9 (C-13), [133.2<sub>4</sub>, 133.2<sub>2</sub>, 133.1, 132.8, 132.7, 132.6, 129.9<sub>5</sub> (2 $\times$ ), 129.9<sub>2</sub> (2 $\times$ ), 129.8<sub>1</sub> (4 $\times$ ), 129.7<sub>6</sub> (4 $\times$ ), 129.6<sub>3</sub>, 129.5<sub>7</sub>, 129.5<sub>1</sub>, 129.4, 129.3 (2 $\times$ ), 128.3<sub>9</sub> (2 $\times$ ), 128.3<sub>5</sub> (2 $\times$ ), 128.3<sub>3</sub> (2 $\times$ ), 128.2 (2 $\times$ ), 128.1 (2 $\times$ ), 128.0 (2 $\times$ ), CH-Bz, C-Bz], 125.9 (C-12), 101.9 (C-1'), 99.7 (C-1''), 99.5 (C-1'''), 89.9 (C-3), 81.7 (C-3'), 81.0 (C-4'), 72.0 (C-4''), 72.0 (C-4'''), 71.9 (C-2''), 71.3 (C-2'''), 71.1 (C-2'), 69.4 (C-3'''), 69.4 (C-3''), 67.7 (C-5'''), 67.6 (C-5''), 67.1 (C-5'), 55.4 (C-5), 52.6 (C-18), 47.9 (C-17), 47.6 (C-9), 42.0 (C-14), 39.5 (C-8), 39.1 (C-19), 39.0 (C-4), 38.8 (C-20), 38.7 (C-1), 36.7 (C-10), 36.6 (C-22), 32.9 (C-7), 30.5 (C-21), 28.4 (C-23), 28.0 (C-15), 25.5 (C-2), 24.2 (C-16), 23.6 (C-27), 23.4 (C-11), 21.2 (C-30), 18.8 (C-6), 18.3 (C-6'), 17.7 (C-6''), 17.6 (C-6'''), 17.0 (C-29), 16.9 (C-26), 16.7 (C-24), 15.5 (C-25). HRMS calcd for  $\text{C}_{90}\text{H}_{106}\text{O}_{21}\text{N}$  [ $\text{M} + \text{NH}_4$ ] $^+$  1536.7252, found 1536.7236.

**3-O- $\alpha$ -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl ursolic acid (9).** Compound 28 (34.4 mg, 23  $\mu\text{mol}$ ) was dissolved in MeOH–DCM (2 : 1, 3 mL) with NaOMe in MeOH (0.5 M, 3 mL) overnight. The reaction was then neutralized to pH 7 with Dowex G26 ( $\text{H}^+$  form), filtered and purified by flash chromatography (9 : 1, DCM–MeOH  $\rightarrow$  26 : 14 : 3 DCM–MeOH– $\text{H}_2\text{O}$ ) to give compound 9 (17 mg, 84%) as a colorless oil,  $R_f = 0.3$  (26 : 14 : 3  $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$ ),  $[\alpha]_{\text{D}}^{20} +12.3$  (c 1.8, 1 : 1,  $\text{CHCl}_3$ –MeOH).  $^1\text{H}$  NMR [400 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$  1 : 1]  $\delta$  5.23 (br s, H-12), 5.00 (br s, 1H, H-1''), 4.88 (br s, 1H, H-1'''), 4.73 (m, 1H, H-1'), 3.96–3.62 (m, 10H, H-2'', H-



2', H-3', H-2'', H-5', H-5''', H-3''', H-5'', H-4', H-3''), 3.46–3.38 (m, 2H, H-4'', H-4'''), 3.11 (dd,  $J = 10.9, 4.7$  Hz, 1H, H-3), 1.32–1.26 (m, 9H, H-6', H-6'', H-6'''), 1.11 (s, 3H, H-27), 0.99–0.94 (m, 9H, H-30, H-25, H-23), 0.88 (d,  $J = 6.4$  Hz, 3H, H-29), 0.85 (s, 3H, H-26), 0.81 (s, 3H, H-24).  $^{13}\text{C}$  NMR [101 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$  1 : 1]  $\delta$  179.7 (C-28), 139.2 (C-13), 125.9 (C-12), 103.5 (C-1'''), 103.3 (C-1'), 102.9 (C-1''), 90.1 (C-3), 80.9 (C-3'), 80.0 (C-4'), 73.2 (C-4'''), 73.0 (C-4''), 71.8 (C-3'''), 71.7 (C-2''), 71.6 (C-2'''), 71.6 (C-3'''), 71.4 (C-2'), 69.9 (C-5''), 69.6 (C-5'''), 68.3 (C-5'), 56.0 (C-5), 53.7 (C-18), 48.2 (C-9), 42.7 (C-14), 40.1 (C-8), 39.9 (C-19), 39.7 (C-20), 39.5 (C-4), 39.2 (C-22), 37.3 (C-1), 37.3 (C-10), 33.7 (C-7), 28.7 (C-15), 28.6 (C-23), 26.0 (C-2), 24.9 (C-16), 23.9 (C-27), 23.9 (C-11), 21.5 (C-30), 18.9 (C-6), 18.2 (C-6'), 17.6 (C-6'''), 17.5 (C-6''), 17.5 (C-26), 17.5 (C-29), 16.9 (C-24), 15.9 (C-25). HRMS: calcd for  $\text{C}_{48}\text{H}_{82}\text{O}_{15}\text{N}$  [ $\text{M} + \text{NH}_4$ ] $^+$  912.5679, found 912.5678.

**3-O-(2,3,4-Tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2))-2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3))-2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4))- $\alpha$ -L-rhamnopyranosyl-28-benzoyl ursolate (29).** Based on the general procedure for inverse Schmidt glycosylation, compound 22 (89 mg, 131  $\mu\text{mol}$ ) and TMSOTf (2.4  $\mu\text{L}$ , 13  $\mu\text{mol}$ ) were dissolved in dry DCM (4 mL) with 4 Å molecular sieves (50 mg) at  $-78^\circ\text{C}$ . After stirring the solution during 30 min, a solution of 2,3,4-tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate 12 (407 mg, 656  $\mu\text{mol}$ ) in dry DCM (2 mL) was added. The reaction mixture was stirred at  $-15^\circ\text{C}$  for 25 min then the reaction was quenched with  $\text{Et}_3\text{N}$  (0.2 equiv.) and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography (hexanes–EtOAc, 5  $\rightarrow$  30%) to afford the expected compound 29 (127 mg, 47%) as a colourless oil.  $R_f = 0.48$  (7 : 3, hexanes–EtOAc);  $[\alpha]_{\text{D}}^{20} +12.3$  (c 1,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07–7.73 (m, 18H, CH-Bz), 7.55–7.06 (m, 32H, CH-Bz, CH-Bn), 6.11–5.94 (m, 5H, H-3''', H-2''', H-3'', H-3''', H-2''), 5.88 (dd,  $J = 3.4, 1.6$  Hz, 1H, H-2'''), 5.78–5.67 (m, 3H, H-4'', H-4''', H-4'''), 5.60 (br s, 1H, H-1''), 5.39 (br s, 1H, H-1'''), 5.38 (br s, 1H, H-1''') 5.26 (t,  $J = 3.5$  Hz, 1H, H-12), 5.11 (d,  $J = 12.5$  Hz, 1H,  $\text{CH}_2$ -Bn), 5.07 (br s, 1H, H-1'), 4.99 (d,  $J = 12.5$  Hz, 1H,  $\text{CH}_2$ -Bn), 4.50 (dq,  $J = 9.7, 6.5$  Hz, 1H, H-5'''), 4.44–4.34 (m, 2H, H-5''', H-5''), 4.32 (dd,  $J = 8.8, 2.6$  Hz, 1H, H-3'), 4.23 (br s, 1H, H-2'), 4.09–3.98 (m, 2H, H-4', H-5'), 3.14 (dd,  $J = 10.5, 4.6$  Hz, 1H, H-3), 2.27 (d,  $J = 11.2$  Hz, 1H, H-18), 1.53 (d,  $J = 5.6$  Hz, 3H, H-6'), 1.43 (d,  $J = 6.2$  Hz, 3H, H-6''), 1.36 (d,  $J = 6.5$  Hz, 3H, H-6'''), 1.35 (d,  $J = 6.1$  Hz, 3H, H-6'''), 1.06 (s, 3H, H-27), 0.97 (s, 3H, H-25), 0.95 (s, 3H, H-23), 0.93 (d,  $J = 6.1$  Hz, 3H, H-30), 0.88 (s, 3H, H-24), 0.86 (d,  $J = 6.3$  Hz, 3H, H-29), 0.73 (br d,  $J = 11.0$  Hz, 1H, H-5), 0.65 (s, 3H, H-26).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  177.3 (C-28), [166.1, 166, 165.9, 165.5, 165.15, 165.11 (2 $\times$ ), 165.1, 164.6 (CO-Bz)], 138.1 (C-13), 136.4 (C-Bn), [133.3, 133.18, 133.12, 132.88, 132.81 (2 $\times$ ), 132.72, 132.69, 132.4, 130.06 (2 $\times$ ), 130.04 (2 $\times$ ), 129.95 (2 $\times$ ), 129.87 (4 $\times$ ), 129.81 (4 $\times$ ), 129.76 (4 $\times$ ), 129.68 (2 $\times$ ), 129.63, 129.54, 129.51 (2 $\times$ ), 129.4, 129.3, 128.4 (4 $\times$ ), 128.37 (2 $\times$ ), 128.33 (2 $\times$ ), 128.2 (2 $\times$ ), 128.18 (4 $\times$ ), 128.13 (2 $\times$ ), 128.1 (4 $\times$ ), 128 (2 $\times$ ), 127.9 (2 $\times$ ) (CH-Bz, C-Bz, CH-Bn)], 125.7 (C-12), 100.7 (C-1'), 100.6 (C-1'''), 99.6 (C-1''), 99.2 (C-1'''), 90.0 (C-3), 80.9 (C-4'), 80.7 (C-3'), 79.5 (C-2'), 72.3 (C-4''), 72.1 (C-4'''), 72.0 (C-4'''), 71.7 (C-2''), 71.6 (C-2'''), 71.2 (C-2'''), 69.7 (C-3'''), 69.6 (C-3''), 69.5 (C-3'''), 67.9 (C-5'), 67.8 (C-5''), 67.6 (C-5'''), 67.5 (C-

5'''), 66.0 (C-7), 55.4 (C-5), 52.9 (C-18), 48.1 (C-17), 47.6 (C-9), 42.0 (C-14), 39.6 (C-8), 39.1 (C-19), 39.1 (C-4), 38.8 (C-20), 38.7 (C-1), 36.7 (C-10), 36.7 (C-22), 33.0 (C-7), 30.7 (C-21), 28.5 (C-23), 28.0 (C-15), 25.7 (C-2), 24.3 (C-16), 23.6 (C-27), 23.3 (C-11), 21.2 (C-30), 18.7 (C-6'), 18.3 (C-6), 18.0 (C-6'''), 17.7 (C-6'''), 17.6 (C-6''), 17.0 (C-29), 17.0 (C-26), 16.8 (C-24), 15.5 (C-25). HRMS calcd for  $\text{C}_{124}\text{H}_{134}\text{O}_{28}\text{N}$  [ $\text{M} + \text{NH}_4$ ] $^+$  2084.90869, found 2084.91553.

**3-O- $\alpha$ -L-Rhamnopyranosyl-(1 $\rightarrow$ 2))- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3))- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4))- $\alpha$ -L-rhamnopyranosyl ursolic acid (10).** A solution of compound 29 (55 mg, 27  $\mu\text{mol}$ ) and 10% Pd/C (2 mg) in dry EtOAc (3 mL) was refluxed and purged with  $\text{H}_2$ . When TLC indicated the disappearance of the starting material, the mixture was filtered over Celite® and concentrated under reduced pressure. The residue was suspended in dry MeOH–DCM (2 : 1, 2 mL), to which a freshly prepared solution of NaOMe (0.5 M, 2 mL) was added. After overnight stirring, the reaction was neutralized to pH 7 with Dowex G-26 ( $\text{H}^+$  form) and filtered. The filtrate was concentrated to dryness and purified by reversed-phase flash chromatography SPE (1 g cartridge,  $\text{H}_2\text{O} : \text{MeOH}$  50  $\rightarrow$  85%), to give 10 (25 mg, 90% over two steps)  $R_f = 0.18$  (26 : 14 : 3  $\text{CHCl}_3$ –MeOH– $\text{H}_2\text{O}$ );  $[\alpha]_{\text{D}}^{20} +36.8$  (c 3.9, 1 : 1,  $\text{CHCl}_3$ –MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$  1 : 1)  $\delta$  5.24 (br s, 1H, H-12), 4.98 (s, 1H, H-1''), 4.92 (s, 1H, H-1'''), 4.86 (s, 1H, H-1'''), 4.85 (s, 1H, H-1'), 3.98 (dd,  $J = 9.8, 2.8$  Hz, 1H, H-3'), 3.94–3.87 (m, 3H, H-2''', H-2'', H-2'), 3.85–3.57 (m, 9H, H-2'', H-5', H-5'', H-3''', H-5''', H-5''', H-3'', H-3''', H-4'), 3.47–3.32 (m, 3H, H-4'', H-4'', H-4'''), 3.11 (t,  $J = 8.4$  Hz, 1H, H-3), 2.20 (br d,  $J = 11.2$  Hz, 1H, H-18), 1.28 (m, 12H, H-6', H-6'', H-6''', H-6'''), 1.11 (s, 3H, H-27), 0.96 (m, 9H, H-30, H-25, H-23), 0.88 (d,  $J = 6.4$  Hz, 3H, H-29), 0.84 (s, 3H, H-26), 0.81 (s, 3H, H-24).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}/\text{CDCl}_3$  1 : 1)  $\delta$  181.5 (C-28), 138.9 (C-13), 126.0 (C-12), 103.6 (C-1'''), 102.9 (C-1'), 102.9 (C-1'''), 101.9 (C-1'), 90.0 (C-3), 80.2 (C-3'), 80.2 (C-4'), 79.1 (C-2'), 73.2 (C-4'''), 73.1 (C-4'''), 72.9 (C-4''), 71.8 (C-3''), 71.7 (C-3'''), 71.6 (C-2''), 71.6 (C-3'''), 71.3 (C-2'''), 71.3 (C-2''), 69.9 (C-5''), 69.9 (C-5'''), 69.6 (C-5'''), 68.3 (C-5'), 55.9 (C-5), 53.6 (C-18), 48.3 (C-17), 48.2 (C-9), 42.7 (C-14), 40.1 (C-8), 39.7 (C-19), 39.6 (C-20), 39.6 (C-4), 39.1 (C-1), 37.5 (C-22), 37.3 (C-10), 33.6 (C-7), 31.2 (C-21), 28.7 (C-23), 28.6 (C-15), 26.1 (C-2), 24.8 (C-16), 23.9 (C-27), 23.8 (C-11), 21.4 (C-30), 18.8 (C-6), 18.3 (C-6''), 17.8 (C-6'''), 17.7 (C-6'), 17.5 (C-6'''), 17.4 (C-26), 17.4 (C-29), 16.8 (C-24), 15.8 (C-25). HRMS calcd for  $\text{C}_{54}\text{H}_{88}\text{O}_{19}\text{Na}$  [ $\text{M} + \text{Na}$ ] $^+$  1063.5812, found 1063.5845.

**Cell culture.** Human skin fibroblasts WS1 (ATCC CRL-1502), human colon adenocarcinoma DLD-1 (ATCC CCL-221), and murine macrophage RAW 264.7 (ATCC TIB-71) were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in a humidified atmosphere at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$ , in Dulbecco's Minimum Essential Medium supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA), 1 $\times$  vitamins, 100 IU of penicillin and 100  $\mu\text{g mL}^{-1}$  streptomycin (Cellgro®, Mediatech, Manassas, VA, USA).

**Cytotoxicity assay.** Human skin fibroblasts (WS1) and colon adenocarcinoma (DLD-1) were seeded in transparent flat-bottom 96-well microplates (Costar, Corning Inc., Lowell, MA, USA) at a density of  $5 \times 10^3$  cells per well in a volume of 100  $\mu\text{L}$  of culture medium. Cells were allowed to adhere for 16 h before



treatment. Cells were then treated with rhamnosides 1–10 in DMSO. The final concentration of DMSO in the culture medium was 0.5% (v/v) to avoid solvent toxicity. After 48 h, we assessed the cytotoxicity using Hoechst test.<sup>38</sup> Fluorescence was measured on an automated Fluoroskan Ascent FL™ plate reader (Labsystems, Milford, MA, USA) using an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Cytotoxicity was expressed as the concentration that inhibited cell growth by 50% (IC<sub>50</sub>).

**Anti-inflammatory assay.** Murine macrophage RAW 264.7 cells were incubated with rhamnosides 1–10 in DMSO and then stimulated with 100 ng mL<sup>-1</sup> LPS and incubated at 37 °C. Final DMSO concentration was maintained under 0.5% to avoid solvent toxicity. N<sup>ω</sup>-Nitro-L-arginine methyl ester hydrochloride (L-NAME), a known iNOS inhibitor, was used as positive control. After 24 h, the cell-free supernatants were collected and NO concentration was immediately determined using the Griess reaction.<sup>39</sup> The absorbance was measured at 550 nm using an automated Varioskan Ascent plate reader (Labsystems, Milford, MA, USA), to quantify the presence of nitrite by comparing with a NaNO<sub>2</sub> standard curve.

## Authors contribution

A. P., B. S., and C. G. designed the experiments. B. S. and J. L. performed the experiments. B. S. and S. L. analyzed the NMR data. B. S., C. G., and J. L. analyzed the data and wrote the paper. A. P. and J. L. secured funding. A. P., J. L., and C. G. directed the students.

## Conflicts of interest

The authors have no conflict of interest to declare.

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