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Synthesis, cytotoxicity, and haemolytic activity of chacotrioside lupane-type neosaponins and their germanicane-type rearrangement products

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ABSTRACT

The concise synthesis, via a stepwise glycosylation approach, of lupeol, betulin and betulinic acid O-glycosides bearing a chacotriosyl moiety at the C-3 position is described. All neosaponins as well as their rearrangement products of the germanicane-type were evaluated in vitro for their anticancer and haemolytic activities. Although betulinic acid and betulin 3β-*O*-chacotriosides were neither cytotoxic nor haemolytic, their rearrangement products allobetulin and 28-oxoallobetulin 3β-*O*-chacotriosides (**9** and **10**) exhibited a cytotoxicity profile up to fourfold superior to betulinic acid against human breast (MCF7) and prostate (PC-3) adenocarcinomas cell lines (IC_{50} = 10–18 μM). One important result was that only chacotriosides featuring non-polar functions at the C-28 position (**6**, **9** and **10**) exerted a haemolytic activity against red blood cells.

The naturally occurring lupane-type triterpenoids lupeol (**1**), betulin (**2**) and betulinic acid (**3**) have been thoroughly investigated during the past years for their promising medicinal properties,[1–5] and particularly their chemopreventive[6] and antitumor[7,8] activities. Consequently, several studies were mainly focused on the preparation of anticancer derivatives of triterpenoids **2** and **3** modified at C-3 and/or C-28 positions.[1,8–11] To increase water solubility of these non-polar cholesterol-like triterpenoids as well as to study the structure–activity relationships, a broad library of mono- and bidesmosidic lupane-type saponins were recently synthesized in our laboratory.[12–15] These molecules have been evaluated for their cytotoxicity against human cancer cells growth and the main observation was that the addition of rhamnose moieties at both C-3 and C-28 positions of triterpenoids **2** and **3** gives anticancer agents many fold stronger than betulinic acid (**3**).[15] Furthermore, it was demonstrated that contrary to the majority of naturally occurring saponins,[16,17] most of the lupane-type glycosides do not exhibit any haemolytic activity (HD_{50} > 100 μM) against red blood cells,[18] which is of a great interest regarding their clinical utilisation as intravenously delivered anticancer agents.

Saponins bearing a 2,4-branched trisaccharide containing rhamnose moieties such as chacotriosides are very attractive for their anticancer activity. Indeed, dioscin, namely diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside, a well-known saponin isolated from several plant species used in traditional oriental medicine, induces apoptosis within cancerous cells[19,20] and exhibits promising antitumor activities.[21–23] Moreover, the apoptosis-inducing activity of solamargine, a solasodine steroidal alkaloid bearing the same chacotriosyl moiety, is correlated with the presence of rhamnose moieties.[24,25] Therefore, we thought that it would be of interest to prepare lupane-type saponins incorporating this particular 2,4-branched trisaccharide. Hence, as shown in Figure 1, we report here the synthesis of lupeol, betulin and betulinic acid monodesmosidic saponins (**6**–**8**) bearing a chacotriosyl moiety at the C-3 position as well as their unexpected germanicane-type rearrangement products (**9** and **10**). The in vitro cytotoxic and haemolytic activities of all synthesized neosaponins are also reported.

In this work, a stepwise glycosylation strategy was chosen rather than a convergent one, in order to obtain exclusively a 1,2-*trans*-glycosidic linkage.[26] Thus, as depicted in Scheme 1, the synthesis began by coupling the lupane-type acceptors lupeol (**1**),[12] 28-*tert*-butyldiphenylsilyl betulin (**11**)[14] or allyl betulinate (**12**)[13] with the donor 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**13**, 1.5 equiv)[12] under the promotion of the Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.1 equiv). After the glycosylation, removal of the benzoyl groups under standard deprotection conditions (0.5 N NaOH, MeOH/THF/H_2O 1:2:1, room temperature) afforded target β-D-glucosides **14**[12] (90%), **15** (72%) and **16** (80%) in good to excellent yields after two steps. Then, the regioselective pivaloylation[27] at both C-6′ and

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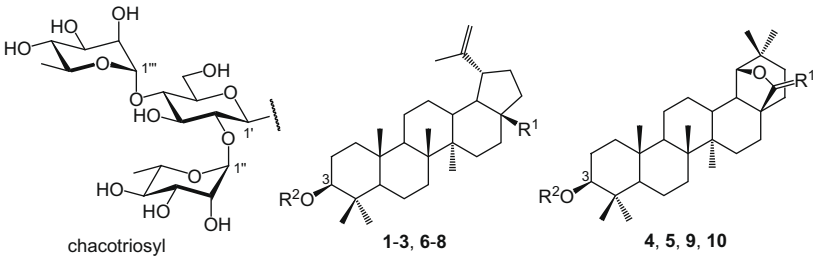
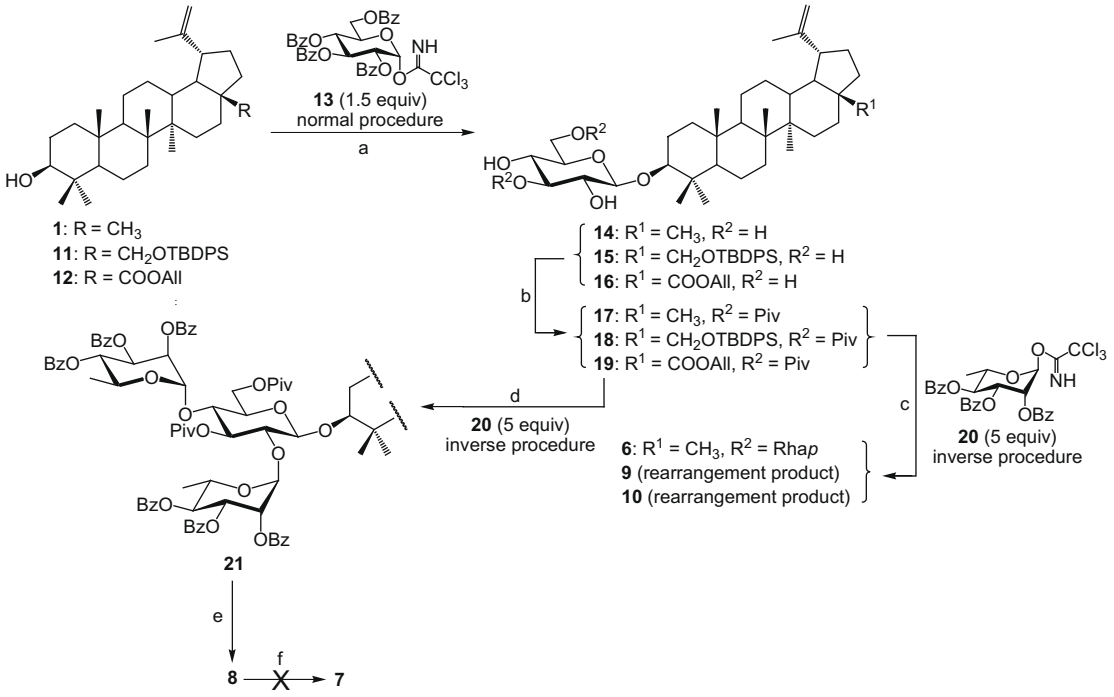


	R^1	R^2
Lupeol (**1**):	CH_3	H
Betulin (**2**):	CH_2OH	H
Betulinic acid (**3**):	COOH	H
Allobetulin (**4**):	H_2	H
28-Oxoallobetulin (**5**):	O	H
6:	CH_3	chacotriosyl
7:	CH_2OH	chacotriosyl
8:	COOH	chacotriosyl
9:	H_2	chacotriosyl
10:	O	chacotriosyl

Figure 1. Triterpenes **1**–**5** and chacotrioside neosaponins **6**–**10**.



Scheme 1. Reagents and conditions: (a) (i) TCA **13** (1.5 equiv), TMSOTf (0.1 equiv), 4 Å MS, CH_2Cl_2, rt, 2 h; (ii) NaOH (0.5 N), MeOH/THF/H_2O 1:2:1, rt, overnight, 90% for **14** (two steps); 72% for **15** (two steps); 80% for **16** (two steps); (b) PivCl (5.1 equiv), Py, 0 °C to rt, 4 h, 60% for **17**; 61% for **18**; 62% for **19**; (c) (i) TCA **20** (5.0 equiv), TMSOTf (0.5 equiv), 4 Å MS, CH_2Cl_2, −10 °C to rt, 3.5–5 h; (ii) NaOH (0.5 N), MeOH/THF/H_2O 1:2:1, rt, three days, 57% for **6** (two steps); 32% for **9** (two steps); 24% for **10** (two steps); (d) TCA **20** (5.0 equiv), TMSOTf (<0.2 equiv), 4 Å MS, CH_2Cl_2, rt, 3 h, 96%; (e) (i) NaOH (0.5 N), MeOH/THF/H_2O 1:1:1, 50 °C, 5 h; (ii) $Pd^0(PPh_3)_4$ (0.3 equiv), PPh_3 (0.6 equiv), pyrrolidine (2.0 equiv), THF, rt, 4 h, 78% (two steps); (f) $LiAlH_4$ (2.6 equiv), THF, reflux, 4 h.

C-3′ positions of the glucosides **14**–**16** using pivaloyl chloride (PivCl) in pyridine (Py) provided, as expected, the protected derivatives **17**–**19** in moderate yields (60–62%). The Schmidt's inverse procedure[28] was chosen in order to synthesize the chacotrioside moiety. This approach gave better yields compare to the normal procedure when two hydroxyl groups are simultaneously glycosylated.[29] Thus, coupling of the acceptor **17** with the donor 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**20**, 5.0 equiv) using TMSOTf (0.5 equiv) at −10 °C to room temperature followed by removal of benzoyl and pivaloyl groups led to the formation of the target chacotrioside lupeol saponin **6** in a moderate yield (57%, two steps). Surprisingly, under the same glycosylation conditions, the germanicane-type rearrangement products allobetulin and 28-oxoallobetulin 3β-*O*-chacotriosides (**9** and **10**) were obtained in 32% and 24% yields, respectively, instead of the expected C-28 protected betulin and betulinic acid 3β-*O*-chacotriosides. This result may be explained by the fact that triterpenes **2** and **3** may undergo a Wagner–Meerwein rearrangement in the presence of an excess of Lewis acids.[13,30,31] Therefore, in light of these results, a separate experiment was performed by glycosylation of the accep-

tor **16** with the donor **20** using a smaller amount of TMSOTf (<0.2 equiv) in the reaction medium. Under these conditions (Scheme 1), the fully protected chacotrioside derivative **21** was prepared in an excellent yield (96%) without any detectable trace of rearrangement products. The target chacotrioside betulinic acid saponin **8** (78%, two steps) was finally obtained after the hydrolysis of benzoyl and pivaloyl groups (0.5 N NaOH, MeOH/THF/H_2O 1:1:1, 50 °C) followed by the subsequent deallylation at the C-28 position.

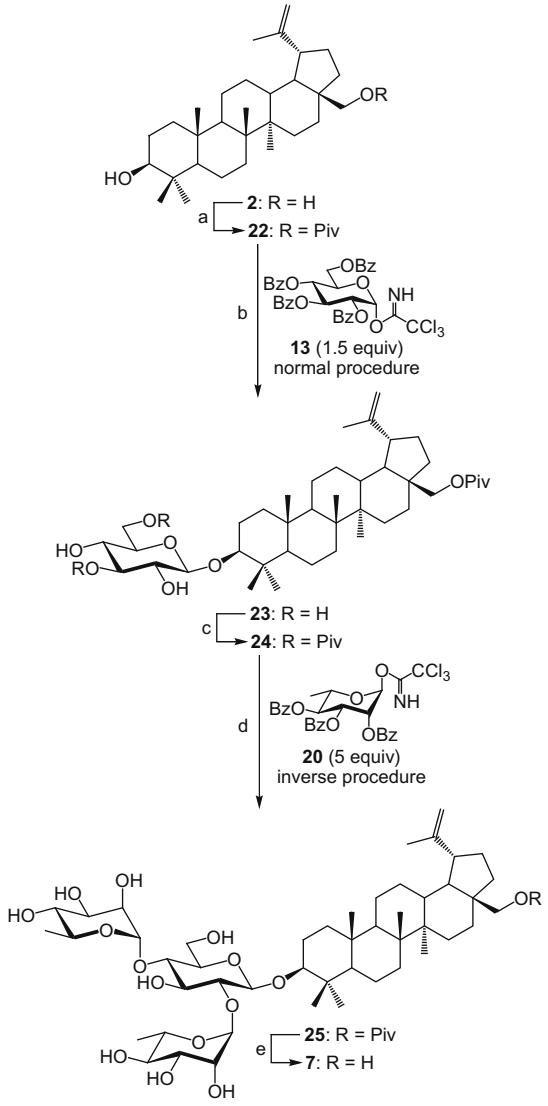
For some unclear reasons, all attempts to prepare the chacotrioside betulin saponin **7** from the glucosidic derivative **18** were unsuccessful (see Table 1). Moreover, trials were made to obtain the target neosaponin **7** by the reduction of the carboxylic acid function of **8** using aluminium lithium hydride ($LiAlH_4$) in refluxing THF.[32] Unfortunately, the reaction resulted in a complex mixture of inseparable products. We next reasoned that using another less hindered protecting group than TBDPS at the C-28 position of betulin (**2**) such as a pivalate ester (Piv) should produce better results. Thus, as depicted in Scheme 2 , 28-*O*-pivaloyl betulin (**22**), which was synthesized in good yield (76%) by treatment of betulin (**2**) with PivCl in pyridine, was glucosylated at the C-3 position to afford **23** (68%, two steps) after deprotection of the benzoyl groups. Regioselective pivaloylation of **23** led to the formation of **24** (60%), which was then simultaneously glycosylated at both C-2′ and C-4′ positions with the TCA sugar donor **20** via the Schmidt's inverse procedure to provide the fully protected chacotriosidic derivative. Unexpectedly, deprotection of benzoyl and pivaloyl groups of the crude product using 0.5 N NaOH at 50 °C afforded 28-*O*-pivaloyl betulin 3β-chacotrioside (**25**) instead of **7**. Thus, in order to cleave this sterically hindered primary pivalate ester, compound **25** was subjected to an alkaline hydrolysis at a higher NaOH concentration (1.5 N) and a longer reflux period (48 h). Under these conditions, the target chacotrioside saponin **7** was finally obtained in a pure and homogeneous form together with an excellent yield (88%). Additionally, it is worth noting that, by analysing the coupling constant values of the anomeric protons, 1,2-*trans*-glycosidic linkages (α-L-Rha*p* and β-D-Glc*p*) were obtained for all the newly synthesized neosaponins (**6**–**10**).[33]

The cytotoxicity of the synthesized neosaponins (**6**–**10**) was evaluated in vitro by the resazurin reduction test[34] against lung carcinoma (A549), colorectal adenocarcinoma (DLD-1), breast adenocarcinoma (MCF7), prostate adenocarcinoma (PC-3) and normal skin fibroblasts (WS1) human cell lines. Betulinic acid (**3**) was used as a positive control.[35] Cytotoxicity results displayed in Table 2 are expressed as the concentration inhibiting 50% of the cell growth (IC_{50}). Additionally, haemolytic activity of neosaponins **6**–**10** was assessed in vitro on sheep red blood cells (erythrocytes). As shown in Table 2, the values are expressed as the concentration inducing 50% haemolysis of erythrocytes (HD_{50}). PBS buffer (pH 7.4) was used as negative control and Sigma–Aldrich® saponin mixture from quillaja bark (20–35% sapogenin) was used as positive control.

Table 1
Attempts to synthesize neosaponin **7** from derivative **18**[a]

Entry	RhaTCA[b] (equiv)	Promoter (equiv)	CH_2Cl_2 (mL/mmol)	Temperature
1	5.0	TMSOTf (1.0)	23	−10 °C to rt
2	5.0	TMSOTf (0.2)	23	rt
3	5.0	TMSOTf (0.2)	23	−10 °C to rt
4	3.0	TMSOTf (0.1)	18	−10 °C to rt
5	5.0	TMSOTf (0.05)	43	−10 °C to rt
6	5.0	$BF_3{\cdot}OEt_2$ (1.0)	28	−40 °C to rt

[a] Reactions were performed overnight at 0.1 mmol scale by using the Schmidt's inverse procedure.
[b] 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranoside trichloroacetimidate (**20**) was used as acceptor.



Scheme 2. Reagents and conditions: (a) PivCl (1.2 equiv), Py, 0 °C to rt, 6 h, 76%; (b) (i) TCA **13** (1.5 equiv), TMSOTf (0.1 equiv), 4 Å MS, CH_2Cl_2, rt, 2 h; (ii) NaOH (0.5 N), MeOH/THF/H_2O 1:2:1, rt, overnight, 68% (two steps); (c) PivCl (5.1 equiv), Py, 0 °C to rt, overnight, 60%; (d) (i) TCA **20** (5.0 equiv), TMSOTf (<0.2 equiv), 4 Å MS, CH_2Cl_2, rt, 3 h; (ii) NaOH (0.5 N), MeOH/THF/H_2O 1:1:1, 50 °C, 5 h, 54% (two steps); (e) NaOH (1.5 N), MeOH/H_2O 2:1, reflux, 48 h, 88%.

The cytotoxic results indicate that the chacotrioside moiety has a negative impact on the anticancer activity of lupane-type triterpenoids (IC_{50} > 50 μmol L^{-1}). These results are in good agreement with previous investigations in which lupane-type saponins bearing highly polar sugar moieties at the C-3 position such as β-D-glucose[12,15] or α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranose[18] were not or only weakly cytotoxic against human cancer cell lines. On the other hand, it is worth noting that allobetulin and 28-oxo-allobetulin chacotriosides (**9** and **10**) exhibited a cytotoxicity profile similar and even stronger than betulinic acid (**3**) with IC_{50} values ranging from 3.8 to 18 μmol L^{-1}. Indeed, neosaponins **9** and **10** were about fourfold more active than betulinic acid (**3**) to inhibit the growth of human prostate adenocarcinoma cell lines (IC_{50} = 10–13 μmol L^{-1}). To our knowledge, it is the first time that such an increase in the anticancer activity of germanicane-type triterpenoids **4** and **5** was reported in the literature.[13] Thus, the results suggest that the beneficial cytotoxic effect of the chacotrioside moiety is highly dependent of the aglycone nature.

Table 2
Cytotoxic (IC_{50}) and haemolytic (HD_{50}) activities of triterpenoids **1**–**5** and chacotrioside neosaponins **6**–**10**

Compound	HD_{50}[a] (μmol L−1)	IC_{50}[a] (μmol L^{-1})				
		A549[b]	DLD-1[c]	MCF7[d]	PC-3[e]	WS1[f]
1	> 100	> 50	> 50	> 50	> 50	> 50
2	> 100	3.8 ± 0.1	6.6 ± 0.3	NT[g]	NT[g]	3.6 ± 0.1
3[h]	> 100	10.3 ± 0.4	15.0 ± 0.3	41 ± 1	40 ± 2	12 ± 1
4	> 100	> 50	> 50	> 50	> 50	> 50
5	> 100	> 50	> 50	> 50	> 50	> 50
6	30 ± 2	> 50	> 50	> 50	> 50	> 50
7	> 100	> 50	> 50	> 50	> 50	> 50
8	> 100	> 50	> 50	> 50	> 50	> 50
9	90 ± 9	14 ± 2	13 ± 2	15 ± 2	13 ± 2	9 ± 1
10	8.0 ± 0.9	13 ± 1	14 ± 1	18 ± 2	10 ± 1	3.8 ± 0.2
PBS	> 100	—	—	—	—	—
Saponin[i]	7.4 ± 0.3	—	—	—	—	—

[a] Mean values ± standard deviation for three independent experiments made in triplicate.
[b] Human lung carcinoma.
[c] Human colorectal adenocarcinoma.
[d] Human breast adenocarcinoma.
[e] Human prostate adenocarcinoma.
[f] Human normal skin fibroblasts.
[g] Not tested.
[h] Betulinic acid (**3**) was used as a positive control for the cytotoxic assay.
[i] Value in μg mL^{-1}. Positive control for the haemolytic assay.

Interestingly, this study shows that the chacotriose moiety increases the haemolytic activity of the less polar triterpenoids, that is, lupeol (**1**), allobetulin (**4**) and 28-oxoallobetulin (**5**), but not for betulin (**2**) and betulinic acid (**3**).[18] Moreover, the presence of an additional carbonyl group (lactone function) on neosaponin **10** increases the haemolytic activity up to 10-fold in comparison with neosaponin **9**. These results corroborate the conclusions of Biao Yu and co-workers[36] who showed in a recent SAR study that the nature of the aglycone strongly affects the haemolytic activities of the chacotrioside saponins. Noteworthy, allobetulin chacotrioside (**9**) is an interesting compound for further in vivo studies since it is weakly haemolytic (HD_{50} = 90 ± 9 μmol L^{-1}) and exhibits a good cytotoxicity profile against cell lines derived from the most prevalent human cancer types. However, saponin **9** was also cytotoxic against human normal fibroblast cell line (WS1).

In conclusion, a series of five saponins of the lupane- and germanicane-type bearing a chacotrioside moiety at the C-3 position were synthesized via a stepwise glycosylation strategy and evaluated for both their cytotoxic and haemolytic activities. Although the lupane-type chacotriosides did not show any cytotoxic activity, allobetulin and 28-oxoallobetulin chacotriosides (**9** and **10**), which are the rearrangement products of triterpenoids **2** and **3**, respectively, exhibited an anticancer activity profile similar and even stronger than betulinic acid (**3**) against human cancer cell lines. On the whole, the results suggest that the cytotoxic and haemolytic activities of saponins containing a chacotrioside moiety at the C-3 position are correlated with the aglycone nature.

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33. *Physical and analytical data for synthesized neosaponins (**6**–**10**): Compound **6**:* $[\alpha]_D^{25}$ − 40.0 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.36 (1H, d, *J* = 0.8 Hz, H-1″), 4.84 (1H, d, *J* = 1.1 Hz, H-1‴), 4.69 (1H, d, *J* = 2.2 Hz, H-29), 4.56 (1H, br s,

H-29), 4.42 (1H, d, J = 7.6 Hz, H-1), 3.97 (1H, m, H-5″), 3.96 (1H, dd, J = 3.3, 1.6 Hz, H-2″), 3.90 (1H, dd, J = 9.4, 6.2 Hz, H-5‴), 3.83 (1H, dd, J = 3.1, 1.8 Hz, H-2‴), 3.80 (1H, m, H-6′), 3.74 (1H, dd, J = 9.5, 3.3 Hz, H-3″), 3.65 (1H, m, H-6′), 3.62 (1H, m, H-3‴), 3.57 (1H, m, H-3′), 3.53 (1H, m, H-4′), 3.44 (1H, m, H-2′), 3.40 (1H, m, H-4‴), 3.38 (1H, m, H-4″), 3.31 (1H, m, H-5′), 3.14 (1H, dd, J = 11.6, 4.3 Hz, H-3), 2.41 (1H, td, J = 11, 5.9 Hz, H-19), 1.68 (3H, s, H-30), 1.26 (3H, d, J = 6.2 Hz, H-6‴), 1.20 (3H, d, J = 6.2 Hz, H-6″), 1.06 (3H, s, H-26), 1.03 (3H, s, H-23), 0.98 (3H, s, H-27), 0.87 (3H, s, H-25), 0.84 (3H, s, H-24), 0.82 (3H, s, H-28); ^{13}C NMR (CD_3OD, 100 MHz): δ 152.0 (C-20), 110.2 (C-29), 105.5 (C-1′), 103.1 (C-1‴), 102.1 (C-1″), 90.4 (C-3), 80.5 (C-4′), 79.2 (C-2′), 78.2 (C-3′), 76.5 (C-5′), 74.0 (C-4″), 73.7 (C-4‴), 72.5 (C-2‴), 72.2 (C-3‴), 72.1 (C-3″), 72.1 (C-2″), 70.8 (C-5‴), 70.0 (C-5″), 62.0 (C-6′), 57.4 (C-5), 51.9 (C-9), 49.6 (C-18), 49.4 (C-19), 44.1 (C-17), 44.0 (C-14), 42.1 (C-8), 41.1 (C-22), 40.4 (C-4), 40.3 (C-1), 39.5 (C-13), 38.1 (C-10), 36.7 (C-16), 35.6 (C-7), 30.9 (C-21), 28.6 (C-15), 28.4 (C-23), 27.4 (C-2), 26.5 (C-12), 22.1 (C-11), 19.6 (C-30), 19.3 (C-6), 18.4 (C-28), 18.0 (C-6″), 17.9 (C-6‴), 17.0 (C-24), 16.9 (C-25), 16.6 (C-26), 15.0 (C-27); HR-ESI-MS m/z 903.5432 $[M+Na]^+$ (calcd for $C_{48}H_{80}O_{14}Na$: 903.5440). *Compound* **7**: $[\alpha]_D^{25}$ + 61.4 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.36 (1H, d, J = 1.3 Hz, H-1″), 4.84 (1H, d, J = 1.5 Hz, H-1‴), 4.68 (1H, br s, H-29), 4.57 (1H, br s, H-29), 4.42 (1H, d, J = 7.8 Hz, H-1′), 3.97 (1H, m, H-5″), 3.96 (1H, dd, J = 3.2, 1.6 Hz, H-2″), 3.91 (1H, dd, J = 9.5, 6.3 Hz, H-5‴), 3.83 (1H, dd, J = 3.2, 1.8 Hz, H-2‴), 3.79 (1H, m, H-6′), 3.74 (1H, m, H-3″), 3.73 (1H, m, H-28), 3.65 (1H, m, H-6′), 3.62 (1H, m, H-3‴), 3.58 (1H, m, H-3′), 3.53 (1H, m, H-4′), 3.44 (1H, m, H-2′), 3.40 (1H, m, H-4‴), 3.38 (1H, m, H-4″), 3.30 (1H, m, H-5′), 3.27 (1H, m, H-28), 3.13 (1H, dd, J = 11.4, 4.1 Hz, H-3), 2.41 (1H, td, J = 10.9, 5.9 Hz, H-19), 1.68 (3H, s, H-30), 1.26 (3H, d, J = 6.2 Hz, H-6‴), 1.20 (3H, d, J = 6.2 Hz, H-6″), 1.06 (3H, s, H-26), 1.03 (3H, s, H-23), 1.00 (3H, s, H-27), 0.86 (3H, s, H-25), 0.84 (3H, s, H-24), 0.73 (1H, d, J = 9.2 Hz, H-5); ^{13}C NMR (CD_3OD, 100 MHz): δ 151.9 (C-20), 110.3 (C-29), 105.5 (C-1′), 103.1 (C-1‴), 102.1 (C-1″), 90.4 (C-3), 80.4 (C-4′), 79.2 (C-2′), 78.2 (C-3′), 76.5 (C-5′), 74.0 (C-4″), 73.7 (C-4‴), 72.5 (C-2‴), 72.2 (C-3‴), 72.1 (C-3″), 72.1 (C-2″), 70.8 (C-5‴), 70.0 (C-5″), 62.0 (C-6′), 60.4 (C-28), 57.4 (C-5), 51.9 (C-9), 50.1 (C-18), 49.4 (C-19), 49.0 (C-17), 43.8 (C-14), 42.2 (C-8), 40.4 (C-4), 40.3 (C-1), 38.7 (C-13), 38.0 (C-10), 35.5 (C-22), 35.1 (C-7), 30.9 (C-21), 30.4 (C-16), 28.4 (C-23), 28.2 (C-15), 27.4 (C-2), 26.7 (C-12), 22.0 (C-11), 19.4 (C-30), 19.3 (C-6), 18.0 (C-6″), 17.9 (C-6‴), 17.0 (C-24), 16.9 (C-25), 16.6 (C-26), 15.2 (C-27); HR-ESI-MS m/z 919.5394 $[M+Na]^+$ (calcd for $C_{48}H_{80}O_{15}Na$: 919.5389). *Compound* **8**: $[\alpha]_D^{25}$ − 53.8 (c 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.36 (1H, d, J = 1.1 Hz, H-1″), 4.84 (1H, d, J = 1.2 Hz, H-1‴), 4.70 (1H, m, H-29), 4.59 (1H, m, H-29), 4.42 (1H, d, J = 7.7 Hz, H-1′), 3.97 (1H, m, H-5″), 3.96 (1H, dd, J = 3.2, 1.4 Hz, H-2″), 3.90 (1H, dd, J = 9.5, 6.2 Hz, H-5‴), 3.83 (1H, dd, J = 3.0, 1.8 Hz, H-2‴), 3.79 (1H, dd, J = 12.0, 1.4 Hz, H-6′), 3.74 (1H, dd, J = 9.5, 3.4 Hz, H-3″), 3.66 (1H, m, H-6′), 3.62 (1H, m, H-3‴), 3.58 (1H, m, H-3′), 3.53 (1H, m, H-4′), 3.44 (1H, m, H-2′), 3.41 (1H, m, H-4‴), 3.38 (1H, m, H-4″), 3.30 (1H, m, H-5′), 3.13 (1H, dd, J = 11.6, 4.0 Hz, H-3), 3.03 (1H, td, J = 10.3, 4.1 Hz, H-19), 2.32 (1H, td, J = 12.6, 3.1 Hz, H-13), 1.69 (3H, s, H-30), 1.26 (3H, d, J = 6.2 Hz, H-6‴), 1.20 (3H, d, J = 6.2 Hz, H-6″), 1.02 (3H, s, H-23), 0.99 (3H, s, H-27), 0.96 (3H, s, H-26), 0.86 (3H, s, H-25), 0.83 (3H, s, H-24), 0.73 (1H, d, J = 9.6 Hz, H-5); ^{13}C NMR (CD_3OD, 100 MHz): δ 180.6 (C-28), 152.3 (C-20), 110.3 (C-29), 105.6 (C-1′), 103.2 (C-1‴), 102.1 (C-1″), 90.6 (C-3), 80.6 (C-4′), 79.3 (C-2′), 78.3 (C-3′), 76.6 (C-5′), 74.1 (C-4″), 73.8 (C-4‴), 72.6 (C-2‴), 72.3 (C-3″), 72.3 (C-3‴), 72.2 (C-2″), 70.9 (C-5‴), 70.1 (C-5″), 62.1 (C-6′), 57.8 (C-17), 57.6 (C-5), 52.2 (C-9), 50.6 (C-18), 48.6 (C-19), 43.7 (C-14), 42.1 (C-8), 40.5 (C-4), 40.5 (C-1), 39.8 (C-13), 38.4 (C-22), 38.2 (C-10), 35.8 (C-7), 33.6 (C-16), 31.9 (C-21), 31.0 (C-15), 28.5 (C-23), 27.5 (C-2), 27.1 (C-12), 22.2 (C-11), 19.7 (C-30), 19.4 (C-6), 18.1 (C-6″), 18.0 (C-6‴), 17.1 (C-24), 17.0 (C-25), 16.8 (C-26), 15.3 (C-27); HR-ESI-MS m/z 933.5179 $[M+Na]^+$ (calcd for $C_{48}H_{78}O_{16}Na$: 933.5182). *Compound* **9**: $[\alpha]_D^{25}$ − 24.0 (c 0.2, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.37 (1H, d, J = 1.4 Hz, H-1″), 4.84 (1H, d, J = 1.5 Hz, H-1‴), 4.43 (1H, d, J = 7.7 Hz, H-1′), 3.97 (1H, m, H-5″), 3.97 (1H, dd, J = 3.2, 1.9 Hz, H-2″), 3.91 (1H, dd, J = 9.5, 6.2 Hz, H-5‴), 3.84 (1H, dd, J = 3.1, 1.8 Hz, H-2‴), 3.80 (1H, m, H-6′), 3.79 (1H, m, H-28), 3.75 (1H, dd, J = 9.6, 3.4 Hz, H-3‴), 3.66 (1H, dd, J = 11.8, 3.7 Hz, H-6′), 3.62 (1H, dd, J = 9.4, 3.2 Hz, H-3″), 3.57 (1H, m, H-3′), 3.55 (1H, s, H-19), 3.54 (1H, m, H-4′), 3.47 (1H, m, H-28), 3.44 (1H, m, H-2′), 3.41 (1H, m, H-4‴), 3.38 (1H, m, H-4″), 3.31 (1H, m, H-5′), 3.16 (1H, dd, J = 11.7, 4.2 Hz, H-3), 1.27 (3H, d, J = 6.2 Hz, H-6‴), 1.21 (3H, d, J = 6.2 Hz, H-6″), 1.03 (3H, s, H-23), 1.02 (3H, s, H-26), 0.96 (3H, s, H-27), 0.92 (3H, s, H-29), 0.89 (3H, s, H-25), 0.84 (3H, s, H-24), 0.83 (3H, s, H-30); ^{13}C NMR (CD_3OD, 100 MHz): δ 105.5 (C-1′), 103.1 (C-1‴), 102.0 (C-1″), 90.4 (C-3), 89.6 (C-19), 80.4 (C-4′), 79.2 (C-2′), 78.2 (C-3′), 76.5 (C-5′), 74.0 (C-4″), 73.7 (C-4‴), 72.5 (C-2‴), 72.2 (C-28), 72.2 (C-3‴), 72.1 (C-3″), 72.0 (C-2″), 70.8 (C-5‴), 70.0 (C-5″), 62.0 (C-6′), 57.6 (C-5), 52.5 (C-9), 48.1 (C-18), 42.7 (C-17), 41.9 (C-14), 41.9 (C-8), 40.5 (C-1), 40.4 (C-4), 38.2 (C-10), 37.6 (C-16), 37.2 (C-20), 35.7 (C-13), 35.1 (C-7), 33.8 (C-21), 29.3 (C-29), 28.4 (C-23), 27.6 (C-15), 27.6 (C-22), 27.4 (C-2), 27.2 (C-12), 24.9 (C-30), 22.3 (C-11), 19.3 (C-6), 18.0 (C-6″), 17.9 (C-6‴), 17.3 (C-25), 17.0 (C-24), 16.3 (C-26), 14.0 (C-27); HR-ESI-MS m/z 919.5380 $[M+Na]^+$ (calcd for $C_{48}H_{80}O_{15}Na$: 919.5389). *Compound* **10**: $[\alpha]_D^{25}$ − 21.5 (c 0.2, MeOH); ^{1}H NMR (CD_3OD, 400 MHz): δ 5.36 (1H, d, J = 1.2 Hz, H-1″), 4.84 (1H, d, J = 1.2 Hz, H-1‴), 4.42 (1H, d, J = 7.7 Hz, H-1′), 4.02 (1H, s, H-19), 3.97 (1H, dd, J = 9.5, 6.2 Hz, H-5″), 3.96 (1H, dd, J = 3.7, 1.5 Hz, H-2″), 3.91 (1H, dd, J = 9.5, 6.3 Hz, H-5‴), 3.83 (1H, dd, J = 3.7, 1.5 Hz, H-2‴), 3.80 (1H, dd, J = 12.1, 1.6 Hz, H-6′), 3.74 (1H, dd, J = 9.5, 3.4 Hz, H-3‴), 3.66 (1H, dd, J = 12.1, 4.1 Hz, H-6′), 3.62 (1H, dd, J = 9.5, 3.4 Hz, H-3″), 3.57 (1H, m, H-3′), 3.53 (1H, m, H-4′), 3.44 (1H, t, J = 8.1 Hz, H-2′), 3.41 (1H, t, J = 9.6 Hz, H-4‴), 3.38 (1H, t, J = 9.6 Hz, H-4″), 3.31 (1H, m, H-5′), 3.15 (1H, dd, J = 11.4, 4.1 Hz, H-3), 2.19 (1H, t, J = 7.4 Hz, H-13), 1.26 (3H, d, J = 6.2 Hz, H-6‴), 1.20 (3H, d, J = 6.2 Hz, H-6″), 1.03 (3H, s, H-23), 1.00 (3H, s, H-30), 0.98 (3H, s, H-29), 0.93 (3H, s, H-26), 0.92 (3H, s, H-27), 0.88 (3H, s, H-25), 0.84 (3H, s, H-24); ^{13}C NMR (CD_3OD, 100 MHz): δ 182.3 (C-28), 105.5 (C-1′), 103.1 (C-1‴), 102.0 (C-1″), 90.3 (C-3), 87.9 (C-19), 80.5 (C-4′), 79.3 (C-2′), 78.2 (C-3′), 76.5 (C-5′), 74.0 (C-4″), 73.7 (C-4‴), 72.5 (C-2‴), 72.2 (C-3‴), 72.2 (C-3″), 72.1 (C-2″), 70.8 (C-5‴), 70.1 (C-5″), 62.0 (C-6′), 57.6 (C-5), 52.7 (C-9), 47.9 (C-18), 47.6 (C-17), 41.8 (C-14), 41.1 (C-8), 40.5 (C-1), 40.4 (C-4), 38.2 (C-10), 37.7 (C-13), 35.0 (C-7), 34.6 (C-20), 33.6 (C-21), 32.8 (C-16), 29.2 (C-30), 29.1 (C-15), 28.4 (C-23), 27.5 (C-22), 27.4 (C-2), 27.3 (C-12), 24.1 (C-29), 22.2 (C-11), 19.2 (C-6), 18.0 (C-6″), 17.9 (C-6‴), 17.3 (C-25), 17.0 (C-24), 16.0 (C-26), 14.1 (C-27); HR-ESI-MS m/z 933.5177 $[M+Na]^+$ (calcd for $C_{48}H_{78}O_{16}Na$: 933.5182).

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