Synthesis and Hemolytic Activity of Some Hederagenin Diglycosides

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Glycosylation of hederagenin with the trichloroacetimidate derivatives of six commercial disaccharides (D-cellobiose, D-lactose, D-maltose, D-melibiose, D-gentiobiose, D-isomaltose) was performed giving the protected saponins in high yields. Deprotection then gave the saponins which were transformed into the corresponding methyl esters. The hemolytic activity of these synthetic hederagenin diglycosides was measured in order to establish structure-activity relationships based on the type and sequence of the attached sugar for the free carboxylic acid and methyl ester saponins.

Keywords: Saponin / Hemolysis / Hederagenin / Glycosylation

Saponins are steroid or triterpenoid glycosides which are widespread in terrestrial and marine plants and possess various biological and pharmacological activities.[1)] One of the first known activities of saponins is their ability to lyse erythrocytes.[1)] This hemolytic activity has long been studied and the origin of the phenomenon is generally ascribed to their amphiphilic properties, but the exact mechanism is not yet known especially when considering the structural diversity of saponins. It has been shown that saponins are capable of interacting with membrane cholesterol[2,3,4)] forming pits and holes which produce a destabilisation of the membrane by a micellar type-arrangement.[5)] Some authors have postulated that the last step consists of an enzymatic deglycosylation that releases the aglycon and produces cell lysis.[6,7)] Aglycons can show weak hemolytic activity but are not solely responsible for cell destruction. While the aglycon is very important in considering structure-activity relationships, the contribution of the sugar moiety is also significant.[8)] Hemolytic activity depends on the nature, number and sequence of the sugars in the saponin.

Our laboratory has long been interested in saponin isolation and identification, and, more recently, in their hemolytic structure-activity relationships. The study of hemolytic activity is a way of gaining insight into how saponins react at the membrane surface. We have compared many saponins with various triterpenoid aglycons and, as Schlösser and Wulff[8)], have established that a polar substitution in ring A and weak polar substitution in rings D and E increases hemolytic activity and that monodesmosidic triterpenoid saponins are generally more active than bidesmosidic ones.[9)] Structure-activity relationships of some synthetic saponins of oleanolic acid,[10,11,12)] methyl oleanolate[13)] and of other triterpenoid or steroid aglycons[14,15)] have been previously established. It is thus known that the effect of the sugar residue on hemolytic activity is not transferable from one aglycon to one another. We wished to complete our study by analyzing the influence of the sugar units on hemolytic activity using hederagenin as the exclusive aglycon. Hederagenin saponins are largely represented in nature and possess many biological activities such as hemolytic, antiviral, antimicrobial, fongicidal, molluscicidal,

or cytotoxic.[1)] With aglycons such as hederagenin, possessing a carboxylic group in position 28, it is known that hemolytic activity is greater when the free acid is esterified as a methyl ester. Unfortunately, no comparative structure-activity relationship have been performed on both the free acid and the methyl ester saponins to establish if the same factors systematically influence hemolytic activity.[8,16)] We thus wished to synthesize the natural saponin 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (cellobiosyl-hederagenin) **1** as well as the non natural 3-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (lactosyl-hederagenin) **2**, 3-*O*-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (maltosyl-hederagenin) **3**, 3-*O*-[α-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (melibiosyl-hederagenin) **4**, 3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (gentiobiosyl-hederagenin) **5**, 3-*O*-[α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (isomaltosyl-hederagenin) **6** (Chart 1), and their corresponding methyl esters (**1a**, **2a**, **3a**, **4a**, **5a**, **6a**). Compound **1** has been isolated from *Fatsia japonica*[17)] and from the leaves of *Barbarea vulgaris* and possesses feeding deterrent activity against the larvae of the insect *Plutella xylostella*.[18)] All of the above compounds were then tested for their hemolytic activity.

Chart 1 near here

Chemistry

Relatively few syntheses of triterpenoid saponins have been reported in the literature in comparison to the large number of those that deal with steroid saponins. This is in part due to the small quantities of aglycon obtained from natural product extraction which is a limiting factor in the preparation of these types of molecules. The key step in saponin synthesis is the glycosylation reaction between the aglycon and the sugar moiety. The disaccharides in our study were previously used in saponin synthesis but with different aglycons[10,12,14,15,19,20)] using

the Koenigs-Knorr method, giving the desired saponins in low yields. We chose to use trichloroacetimidates[21] as donors in our glycosylation reactions, this being a highly efficient method for coupling as reported by Biao Yu et al. for the synthesis of oleanolic acid saponins[22,23] and by us for the synthesis of α-hederin.[24]

Commercial disaccharides were first perbenzoylated. Bromination with 33% HBr/HOAc at 0 °C overnight, hydrolysis of the anomeric bromide with silver carbonate in the presence of acetone/H_2O, followed by activation of the anomeric position with trichloroacetonitrile and DBU gave the desired compounds **7**, **8**, **9**, **10** in 69%, 63%, 86% and 68% yields respectively over 4 steps (Chart 2).

Chart 2 near here

2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **11** was prepared from D-amygdalin as shown in Chart 3.

Chart 3 near here

2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **12** was also synthesized from D-amygdalin as previously reported in the literature.[25]

Glycosylation of the different trichloroacetimidates with the suitably protected hederagenin derivative **13**[24] in the presence of a catalytic amount of TMSOTf at –20 °C first gave a mixture of the orthoester and the desired β product, and this inspite of the use of a benzoate group in position 2. To avoid this problem, three successive amounts of TMSOTf (0.05 eq) were added and the reaction was warmed to room temperature overnight. In this way,

coupling with trichloroacetimidates **7**, **8**, **9**, **10**, **11**, and **12** gave exclusively the desired β products in 70%, 71%, 70%, 75%, 74% and 67% yields respectively (Chart 4).

Total saponin deprotection proceeded in two steps. First, removal of the benzoate esters with 3% KOH/MeOH followed by removal of the allyl with tetrakistriphenylphosphine in the presence of piperidine, giving access to the cellobiosyl **1**, lactosyl **2**, maltosyl **3**, melibiosyl **4**, gentiobiosyl **5** and isomaltosyl **6** hederagenin saponins in 81%, 71%, 78%, 75%, 88% and 74% yields respectively. A fraction of each of these saponins was then treated with diazomethane giving the methyl esters **1a**-**6a** quantitatively (Chart 4).

Chart 4 near here

Hemolytic activity

Previously reported structure-activity relationships of synthetic galactosyl-glucosides and diglucosides saponins of oleanolic acid and methyl oleanolate are contradictory as to the influence of the sugar moiety.[10),12),13)] The hemolytic activity of the hederagenin saponins (**1**-**6**) and methyl hederagenate saponins (**1a**-**6a**) was measured in order to evaluate the influence of the position of the second sugar in relation to the first one ((1→4) or (1→6) linkage), the configuration of the anomeric center of the second sugar residue (α or β), and the type of terminal sugar (D-glucose or D-galactose). The results are reported in Table 1. The percentage of hemolysis was determined with a 10% sheep erythrocyte suspension. Free carboxylic acid saponins **1**–**6** are less active than their corresponding methyl ester **1a**–**6a** except for the maltosyl saponin **3** which is more active than its methyl ester **3a**. The saponins **4**, **5** and **6** are totally inactive at the tested concentrations. The hemolytic activity of their corresponding

methyl esters **4a**–**6a** begins at low concentration but are unable to reach 100% of hemolysis even at the highest concentrations.

Table 1 near here

For the free acid saponins (Figure 1), hemolytic activity is only observed when the second sugar unit is linked in position 4 to the first one (compounds **1**–**3**). This is similar to the result observed by Seebacher et al. for an oleanolic acid aglycon.[12] In addition, the β configuration of the anomeric center between the two sugars (**1**, **2**) is favored over the α one (**3**), and a terminal glucose (equatorial 4-OH) (**1**) slightly increases the activity as compared to a galactose (axial 4-OH) (**2**).

For the methyl ester saponins (Figure 2), substitution in position 4 of the first sugar (**1a**, **3a**) is again favored as opposed to position 6 (**5a**, **6a** respectively). The β configuration is also favored between sugars as observed when comparing the activity of **1a** and **5a** with **3a** and **6a** respectively. In contrast to the results obtained in the free acid saponins, the presence of a terminal galactose (axial 4-OH) (**2a**, **4a**) is favored over a terminal glucose (equatorial 4-OH) (**1a**, **6a**). This phenomena has previously been observed in oleanolic acid and methyl oleanolate saponins but with an opposite effect : a terminal galactose was more active in the free acid saponins. The influence of the terminal sugar seems to have more importance for the methyl ester saponins as a terminal galactose with a (1→6) linkage (**4a**), is more active than a terminal glucose with a (1→4) linkage (**3a**), both saponins having the α anomeric configuration between sugars. The best example of all of the factors united for good hemolytic activity is compound **2a**. The terminal sugar is a galactose (axial 4-OH) with the β configuration linked to the first sugar in position 4. Compound **6a** unites all of the negative

effects : the terminal sugar is a glucose (equatorial 4-OH) with an α configuration linked in position 6 to the first sugar.

Figure 1 near here

Figure 2 near here

Conclusion

Six hederagenin saponins and their methyl esters were easily synthesized from commercial disaccharides sources in good yields by the trichloroacetimidate method. As previously observed, the saponin methyl esters are more active than the free carboxylic acid saponins. The position, the configuration of the interglycosidic linkage and the type of sugars involved does have an influence on hemolytic activity. In the case of both methyl ester and free acid saponins, our study showed that a (1→4) linkage between the sugars increases hemolytic activity as compared to a (1→6) linkage, and that the β configuration of the terminal sugar is also favored. The situation is more complicated when considering the terminal sugar. In our study, a galactose residue is preferred in the case of methyl ester saponins, and a glucose residue is preferred for the free acid saponins. Further studies are in progress in order to determine if certain sugar residues are preferred in the free acid saponin as opposed to the methyl ester saponin, and to understand why there is a difference in hemolytic activity between these two types of glycoconjugates.

Acknowledgements

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Experimental

Chemistry All chemicals were reagent grade and used as supplied unless otherwise noted. Dichloromethane (CH_2Cl_2) and triethylamine were refluxed over calcium hydride and distilled prior to use. All reactions were performed under an Argon atmosphere unless otherwise indicated. Analytical thin-layer chromatography (TLC) was performed on E. Merck Silica Gel 60 F_{254} plates. Compounds were visualized by dipping in an anisaldehyde solution in ethanol and heating. Column chromatography was performed using E. Merck Geduran Silica Gel Si 60 (40-60 μM). Optical rotations were recorded at 21 °C with a Perkin-Elmer 241 polarimeter. ESI-MS were recorded with a Thermofinnigan quadripolar mass spectrometer with positive ion data collected automatically. NMR spectra were obtained using a Bruker Avance DRX 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C). Elemental analyses were performed on a Perkin-Elmer CHN 2400.

Preparation of 2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*α*-D-glucopyranosyl trichloroacetimidate (7). General Method In a typical experiment, benzoyl chloride (2.6 ml, 30.5 mmol, 11 eq) was slowly added to D-cellobiose (1.0 g, 2.8 mmol) in pyridine (11.8 ml) at 0° C. The reaction was stirred for 24h at room temperature, and then heated to 80° C for 3 hours. After cooling, the solvent was evaporated and the residue taken up in CH_2Cl_2 and washed with H_2O, 1M HCl, $NaHCO_3$ (sat), and NaCl (sat). The dried solution (Na_2SO_4) was then evaporated. The crude benzoylated sugar was dissolved in CH_2Cl_2 (6.0 ml), and cooled to 0° C before adding a solution of HBr (33%) in AcOH (6.0 mL). After stirring overnight at 4° C, the reaction was diluted with CH_2Cl_2 and washed with H_2O, $NaHCO_3$ (sat.) and NaCl (sat.). The solution was dried (Na_2SO_4) and evaporated. The crude bromide was then dissolved in a mixture of acetone/H_2O (1:1) (25.0 ml) and Ag_2CO_3 (2.3 g, 8.3 mmol, 3 eq) was added. The reaction was stirred at room temperature for 4 days. Ethyl acetate was then added and the reaction mixture was washed with H_2O, $NaHCO_3$ (sat.) and

NaCl (sat.). The dried solution (Na_2SO_4) was then evaporated and the residue was dissolved in CH_2Cl_2 (47.0 ml). Trichloroacetonitrile (1.4 ml, 13.9 mmol, 5 eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene DBU (3 drops) were added, and the reaction was stirred overnight. The solvent was evaporated to give a crude oil which was purified by column chromatography (cyclohexane/EtOAc, 8:2 to 7:3) to give 2.32 g (69%) of trichloroacetimidate **7**. R_f = 0.40 (cyclohexane/EtOAc, 6:4). $[\alpha]_D$: +53.4 (*c*=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 3.89 (1H, dt, *J* = 4.8, 3.3 Hz, H-5Glc'), 3.94 (1H, dd, *J* = 12.0, 5.1, H-6Glc'), 4.13 (1H, dd, *J* = 12.0, 3.3 Hz, H-6Glc'), 4.38 (2H, m, H-4Glc, H-5Glc), 4.56 (1H, dd, *J* = 12.3, 3.3 Hz, H-6Glc), 4.66 (1H, dd, *J* = 12.5, 1.3 Hz, H-6Glc), 5.08 (1H, d, *J* = 7.9 Hz, H-1Glc'), 5.49 (1H, t, *J* = 9.6 Hz, H-4Glc'), 5.55 (1H, dd, *J* = 10.1, 3.8 Hz, H-2Glc), 5.60 (1H, dd, *J* = 9.7, 7.9 Hz, H-2Glc'), 5.82 (1H, t, *J* = 9.6 Hz, H-3Glc'), 6.21 (1H, dd, *J* = 9.8, 8.8 Hz, H-3Glc), 6.76 (1H, d, *J* = 3.7 Hz, H-1Glc), 7.29-7.64 (21H, m, Ar-H), 7.81 (2H, dd, *J* = 8.4, 1.2 Hz, Ar-H), 7.83 (2H, dd, *J* = 8.5, 1.2 Hz, Ar-H), 7.99 (6H, m, Ar-H), 8.04 (2H, dd, *J* = 8.3, 1.2 Hz, Ar-H), 8.08 (2H, dd, *J* = 8.4, 1.1 Hz, Ar-H), 8.61 (1H, s, N*H*). ^{13}C-NMR ($CDCl_3$) δ: 61.8 (C-6Glc), 62.6 (C-6Glc'), 69.3 (C-4Glc'), 70.1 (C-3Glc), 70.7 (C-2Glc), 71.3 (C-5Glc), 72.0 (C-2Glc'), 72.4 (C-5Glc'), 72.9 (C-3Glc'), 75.9 (C-4Glc), 92.9 (C-1Glc), 101.2 (C-1Glc'), 128.2 (CH), 128.3 (CH), 128.3 (CH), 128.4 (CH), 128.5 (C), 128.6 (C), 129.4 (C), 129.4 (C), 129.5 (CH), 129.6 (CH), 129.7 (CH), 129.9 (CH), 133.2 (CH), 133.3 (CH), 133.4 (CH), 133.5 (CH), 160.6 (*C*=NH), 164.8 (CO), 164.9 (CO), 165.1 (CO), 165.5 (CO), 165.6 (CO). *Anal.* Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15; N 1.15. Found: C, 62.10; H, 3.97; N, 1.11.

2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (8). This compound was prepared using the general method described above. Reaction of D-lactose (1.0 g, 2.8 mmol) gave 2.10 g (63%) of the corresponding imidate **8**. R_f = 0.50 (cyclohexane/EtOAc, 6:4). $[\alpha]_D$: +62.1 (*c*=1.0, $CHCl_3$).

Selected NMR data: ^{1}H-NMR ($CDCl_3$) δ: 3.78 (1H, dd, J = 11.2, 7.3 Hz, H-6Gal), 3.88 (1H, dd, J = 11.2, 6.3 Hz, H-6Gal), 3.96 (1H, m, H-5Gal), 4.40 (2H, m, H-4Glc, H-5Glc), 4.61 (2H, m, 2 × H-6Glc), 5.00 (1H, d, J = 7.9 Hz, H-1Gal), 5.45 (1H, dd, J = 10.3, 3.3 Hz, H-3Gal), 5.60 (1H, dd, J = 10.2, 3.8 Hz, H-2Glc), 5.81 (2H, m, H-2Gal, H-4Gal), 6.22 (1H, t, J = 9.7 Hz, H-3Glc), 6.77 (1H, d, J = 3.7 Hz, H-1Glc), 8.62 (s, 1H, N*H*). ^{13}C-NMR ($CDCl_3$) δ: 61.0 (C-6Glc'), 61.9 (C-6Gal), 67.5 (C-4Gal), 70.0 (C-2Gal), 70.3 (C-3Glc), 70.5 (C-2Glc), 71.4 (C-5Glc), 71.5 (C-5Gal), 71.9 (C-3Gal), 75.6 (C-4Glc), 93.1 (C-1Glc), 101.3 (C-1Gal), 160.7 (*C*=NH). *Anal.* Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15; N 1.15. Found: C, 62.23; H, 4.52; N, 1.09.

2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (9). This compound was prepared using the general method described above. Reaction of D-maltose (1.0 g, 2.8 mmol) gave 2.60 g (86%) of the corresponding imidate **9**. R_f = 0.47 (cyclohexane/EtOAc, 6:4). $[\alpha]_D$: +88.1 (c=1.0, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) δ: 4.30 (1H, dd, J = 12.5, 3.2 Hz, H-6Glc'), 4.43 (1H, dd, J = 12.4, 2.3 Hz, H-6Glc'), 4.48 (1H, m, H-5Glc'), 4.59 (2H, m, H-4Glc, H-5Glc), 4.82 (1H, d, J = 11.5 Hz, H-6Glc), 4.91 (1H, d, J = 12.1 Hz, H-6Glc), 5.33 (1H, dd, J = 10.4, 3.8 Hz, H-2Glc'), 5.42 (1H, dd, J = 10.0, 3.4 Hz, H-2Glc), 5.70 (1H, t, J = 9.8 Hz, H-4Glc'), 5.83 (1H, d, J = 3.8 Hz, H-1Glc'), 6.14 (1H, t, J = 10.0 Hz, H-3Glc'), 6.19 (1H, t, J = 8.7 Hz, H-3Glc), 6.75 (1H, d, J = 3.3 Hz, H-1Glc), 8.62 (1H, s, N*H*). ^{13}C-NMR ($CDCl_3$) δ: 62.4 (C-6Glc'), 62.9 (C-6Glc), 69.0 (C-4Glc'), 69.2 (C-5Glc'), 69.9 (C-3Glc'), 70.7 (C-2Glc'), 71.0 (C-2Glc), 71.2 (C-5Glc), 71.8 (C-3Glc), 72.6 (C-4Glc), 92.9 (C-1Glc), 96.4 (C-1Glc'), 160.5 (*C*=NH). *Anal.* Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15; N 1.15. Found: C, 62.35; H, 4.40; N, 1.09.

2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (10). This compound was prepared using the general method described above. Reaction of D-maltose (1.0 g, 2.8 mmol) gave 2.29 g (68%) of the corresponding imidate **10**. R_f = 0.53 (cyclohexane/EtOAc, 6:4). $[\alpha]_D$: +118.0 (*c*=1.0, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) δ: 3.74 (1H, d, *J* = 9.8 Hz, H-6Glc), 4.08 (1H, dd, *J* = 10.9, 6.7 Hz, H-6Glc), 4.50 (2H, m, H-5Glc, H-6Gal), 4.58 (1H, dd, *J* = 11.5, 4.8 Hz, H-6Gal), 4.94 (1H, m, H-5Gal), 5.30 (1H, dd, *J* = 10.1, 3.8 Hz, H-2Glc), 5.47 (1H, t, *J* = 10.1 Hz, H-4Glc), 5.51 (1H, d, *J* = 3.5 Hz, H-1Gal), 5.78 (1H, dd, *J* = 10.6, 3.5 Hz, H-2Gal), 6.01 (1H, dd, *J* = 10.6, 3.3 Hz, H-3Gal), 6.14 (1H, d, *J* = 3.0 Hz, H-4Gal), 6.23 (1H, t, *J* = 9.9 Hz, H-3Glc), 6.66 (1H, d, *J* = 3.8 Hz, H-1Glc), 9.16 (s, 1H, N*H*). ^{13}C-NMR ($CDCl_3$) δ: 62.9 (C-6Gal), 65.0 (C-6Glc), 67.2 (C-5Gal), 68.6 (C-4Glc), 68.7 (C-3Gal), 68.9 (C-2Gal), 69.5 (C-4Gal), 70.3 (C-3Glc), 70.5 (C-2Glc), 70.1 (C-5Glc), 92.7 (C-1Glc), 95.8 (C-1Gal), 160.0 (*C*=NH). *Anal.* Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15; N 1.15. Found: C, 62.16; H, 4.10; N, 1.11.

2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (11). Benzoyl chloride (1.78 ml, 15.3 mmol, 14 eq) was slowly added to D-gentiobiose (0.500 g, 1.09 mmol) in pyridine (20 ml) at 0° C. The reaction was stirred for 24h at room temperature, and then heated to 80° C for 3 hours. After cooling, the solvent was evaporated and the residue taken up in CH_2Cl_2 and washed with H_2O, 1M HCl, $NaHCO_3$ (sat), and NaCl (sat). The dried solution (Na_2SO_4) was then evaporated. The crude benzoylated sugar was taken up in acetone (28 ml) and ammonium formiate (0.331 g, 5.2 mmol, 4.8 eq) and Pd/C (0.600 g) were added. The reaction was heated to reflux for 6 h. After cooling, the reaction was diluted with EtOAc and washed with 1M HCl, $NaHCO_3$ (sat), and NaCl (sat). The dried solution (Na_2SO_4) was then evaporated. Major impurities were removed by rapid column chromatography (cyclohexane/EtOAc, 3:1). The alcohol obtained was taken

up in CH_2Cl_2 (4 ml). Trichloroacetonitrile (0.400 ml, 4.0 mmol, 5 eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene DBU (3 drops) were added, and the reaction was stirred overnight. The solvent was evaporated to give a crude oil which was purified by column chromatography (cyclohexane/EtOAc, 4:1) to give 0.73 g (60%) of trichloroacetimidate **11**. R_f = 0.47 (cyclohexane/EtOAc, 6:4). $[\alpha]_D$: +29.6 (*c*=1.0, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) δ: 3.90 (1H, dd, *J* = 11.7, 6.1 Hz, H-6Glc), 4.18 (1H, dd, *J* = 11.6, 1.8 Hz, H-6Glc), 4.20 (1H, m, H-5Glc'), 4.51 (1H, dd, *J* = 12.1, 5.3 Hz, H-6Glc'), 4.51 (1H, m, H-5Glc), 4.65 (1H, dd, *J* = 12.2, 3.2 Hz, H-6Glc'), 5.06 (1H, d, *J* = 7.8 Hz, H-1Glc'), 5.42 (1H, dd, *J* = 10.2, 3.6 Hz, H-2Glc), 5.53 (1H, t, *J* = 10.0 Hz, H-4Glc), 5.57 (1H, dd, *J* = 9.7, 7.8 Hz, H-2Glc'), 5.67 (1H, t, *J* = 9.7 Hz, H-4Glc'), 5.94 (1H, t, *J* = 9.6 Hz, H-3Glc'), 6.22 (1H, t, *J* = 9.9 Hz, H-3Glc), 6.72 (1H, d, *J* = 3.6 Hz, H-1Glc), 8.43 (1H, s, N*H*). ^{13}C-NMR ($CDCl_3$) δ: 63.0 (C-6Glc'), 67.3 (C-6Glc), 68.7 (C-4Glc), 69.7 (C-4Glc'), 70.0 (C-3Glc), 70.6 (C-2Glc), 71.7 (C-2Glc'), 72.0 (C-5Glc), 72.2 (C-5Glc'), 72.9 (C-3Glc'), 92.9 (C-1Glc), 100.8 (C-1Glc'), 160.3 (*C*=NH). *Anal.* Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15; N 1.15. Found: C, 62.14; H, 4.13; N, 1.04.

2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (12). This compound was prepared from amygdalin as previously described.[25)]

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl]-23-*O*-benzoylhederagenate (14). General Method In a typical experiment, alcohol **13** (0.370 g, 0.6 mmol), trichloroacetimidate **7** (1.10 g, 0.90 mmol, 1.5 eq) and 4 Å powdered molecular sieves (2.2 g) were stirred for 1h at room temperature in dry CH_2Cl_2 (9 ml). The mixture was cooled to –20 °C for 30 minutes followed by the dropwise addition of a

0.1 M solution of TMSOTf in CH_2Cl_2 (0.300 ml, 0.03 mmol, 0.05 eq). After 2h more TMSOTf was added to the reaction in 30 minute intervals (3 × 0.300 ml). The reaction was then left to warm to room temperature overnight. The next morning, the reaction was quenched with triethylamine, filtered through Celite and evaporated. Purification by column chromatography (toluene/EtOAc, 99:1 to 97:3) gave 0.77 g (70%) of **14** as a white amorphous solid. R_f = 0.39 (toluene/EtOAc, 9:1). $[\alpha]_D$: +41.4 (*c*=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s, H-24), 0.70 (3H, s, H-26), 0.88 (3H, s, H-25), 0.95 (3H, s, H-29), 0.97 (3H, s, H-30), 1.01 (3H, s, H-27), 1.05-2.00 (22H, m, H-1, H-2, H-5, H-6, H-7, H-9, H-11, H-15, H-16, H-19, H-21, H-22), 2.91 (1H, dd, *J* = 13.5, 3.7 Hz, H-18), 3.53 (1H, dd, *J* = 11.7, 4.6 Hz, H-3), 3.75 (1H, dd, *J* = 12.0, 5.4 Hz, H-6Glc'), 3.86 (3H, m, H-23, H-5Glc, H-5Glc'), 3.95 (1H, d, *J* = 11.4 Hz, H-23), 4.07 (1H, dd, *J* = 12.1, 3.0 Hz, H-6Glc'), 4.18 (1H, t, *J* = 9.5 Hz, H-4Glc), 4.54 (3H, m, H-6Glc, C*H*$_2$CH=CH$_2$), 4.62 (1H, dd, *J* = 11.8, 2.0 Hz, H-6Glc), 4.80 (1H, d, *J* = 7.9 Hz, H-1Glc), 4.98 (1H, d, *J* = 7.9 Hz, H-1Glc'), 5.23 (1H, dd, *J* = 10.4, 1.3 Hz, CH$_2$CH=C*H*$_2$), 5.32 (1H, s, H-12), 5.34 (1H, m, CH$_2$CH=C*H*$_2$), 5.43 (1H, t, *J* = 9.7 Hz, H-4Glc'), 5.50 (1H, dd, *J* = 9.8, 8.0 Hz, H-2Glc), 5.55 (1H, dd, *J* = 9.8, 8.0 Hz, H-2Glc'), 5.79 (1H, t, *J* = 9.6 Hz, H-3Glc'), 5.82 (1H, t, *J* = 9.6 Hz, H-3Glc), 5.92 (1H, m, CH$_2$C*H*=CH$_2$), 7.23-7.51 (22H, m, Ar-H), 7.60 (3H, m, Ar-H), 7.81 (4H, m, Ar-H), 7.98 (11H, m, Ar-H). ^{13}C-NMR ($CDCl_3$) δ: 12.6 (C-24), 15.4 (C-25), 16.9 (C-26), 17.9 (C-6), 22.9 (C-16), 23.3 (C-11), 23.6 (C-30), 25.1 (C-2), 25.3 (C-27), 27.4 (C-15), 30.7 (C-20), 32.3 (C-22), 32.3 (C-7), 33.1 (C-29), 33.8 (C-21), 36.3 (C-10), 37.9 (C-1), 39.2 (C-8), 41.4 (C-18), 41.5 (C-14), 42.0 (C-4), 45.8 (C-19), 46.7 (C-17), 47.9 (C-9, C-5), 62.5 (C-6Glc), 62.5 (C-6Glc'), 64.8 (*C*H$_2$CH=CH$_2$), 65.0 (C-23), 69.3 (C-4Glc'), 71.9 (C-2Glc), 72.0 (C-2Glc'), 72.4 (C-5Glc), 72.8 (C-3Glc'), 72.8 (C-5Glc'), 72.9 (C-3Glc), 77.2 (C-4Glc), 83.2 (C-3), 101.0 (C-1Glc'), 102.4 (C-1Glc), 117.7 (CH$_2$CH=*C*H$_2$), 122.2 (C-12), 128.2 (CH), 128.3 (CH), 128.4 (CH), 128.6 (C), 128.8 (C), 129.4 (CH), 129.4 (C), 129.5 (CH), 129.6 (CH), 129.7 (CH), 129.8 (CH), 130.3 (C), 132.5 (CH), 132.8 (CH), 133.0 (CH), 133.1 (CH), 133.3 (CH), 143.7 (C-13), 164.7 (CO), 164.9 (CO), 165.2 (CO), 165.3 (CO), 165.5 (CO), 165.6

(CO), 177.2 (C-28). ESI-MS *m/z* : 1670 $[M+2H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.61; H, 5.99.

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyl]-23-*O*-benzoylhederagenate (15). This compound was prepared in 71% yield using the general glycosylation method described above. R_f = 0.39 (toluene/EtOAc, 9:1). $[\alpha]_D$: +58.5 (*c*=1.0, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) *δ*: 0.66 (3H, s, H-24), 0.71 (3H, s, H-26), 0.89 (3H, s, H-25), 0.95 (3H, s, H-29), 0.97 (3H, s, H-30), 1.02 (3H, s, H-27), 2.91 (1H, dd, *J* = 13.8, 3.5 Hz, H-18), 3.55 (1H, dd, *J* = 11.6, 4.3 Hz, H-3), 3.69 (1H, dd, *J* = 11.6, 6.9 Hz, H-6Gal), 3.75 (1H, dd, *J* = 11.5, 6.8 Hz, H-6Gal), 3.86 (1H, m, H-5Glc), 3.91 (2H, m, 2 × H-23), 3.95 (1H, t, *J* = 6.6 Hz, H-5Gal), 4.21 (1H, t, *J* = 9.4 Hz, H-4Glc), 4.57 (4H, m, 2 × H-6Glc, C*H*$_2$CH=CH$_2$), 4.82 (1H, d, *J* = 7.9 Hz, H-1Glc), 4.91 (1H, d, *J* = 7.9 Hz, H-1Gal), 5.32 (1H, m, H-12), 5.43 (1H, dd, *J* = 10.3, 3.0 Hz, H-3Gal), 5.55 (1H, dd, *J* = 9.7, 8.0 Hz, H-2Glc), 5.76 (1H, dd, *J* = 10.1, 8.2 Hz, 1H, H-2Gal), 5.78 (1H, m, H-4Gal), 5.84 (1H, t, *J* = 9.2 Hz, H-3Glc). ^{13}C-NMR ($CDCl_3$) *δ*: 61.0 (C-6Gal), 62.5 (C-6Glc), 67.5 (C-4Gal), 69.9 (C-2Gal), 71.3 (C-5Gal), 71.7 (C-3Gal), 71.8 (C-2Glc), 72.8 (C-5Glc), 73.0 (C-3Glc), 76.8 (C-4Glc), 101.1 (C-1Gal), 102.6 (C-1Glc). ESI-MS *m/z* : 1670 $[M+2H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.53; H, 6.55.

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-*α*-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyl]-23-*O*-benzoylhederagenate (16). This compound was prepared in 70% yield using the general glycosylation method described above. R_f = 0.48 (toluene/EtOAc, 9:1). $[\alpha]_D$: +64.9 (*c*=1.0, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) *δ*: 0.65 (3H, s, H-24), 0.71 (3H, s, H-26), 0.90 (3H, s, H-25), 0.96 (3H, s, H-29), 0.99 (3H, s, H-30), 1.01 (3H, s, H-27), 2.92 (1H, dd, *J* = 13.7, 4.0 Hz, H-18), 3.60 (1H, dd, *J* = 11.7, 4.6 Hz, H-3), 3.82 (1H, d, *J* = 11.5 Hz,

H-23), 3.98 (1H, d, *J* = 11.4 Hz, H-23), 4.13 (1H, ddd, *J* = 9.0, 5.9, 2.5 Hz, H-5Glc), 4.42 (1H, m, H-6Glc'), 4.43 (1H, t, *J* = 9.4 Hz, H-4Glc), 4.54 (4H, m, H-6Glc', H-5Glc', C*H*$_2$CH=CH$_2$), 4.80 (1H, dd, *J* = 11.7, 6.1 Hz, H-6Glc), 4.89 (1H, d, *J* = 7.8 Hz, H-1Glc), 4.96 (1H, dd, *J* = 11.7, 2.5 Hz, H-6Glc), 5.31 (1H, dd, *J* = 10.5, 3.9 Hz, H-2Glc'), 5.34 (2H, m, H-12, CH$_2$CH=C*H*$_2$), 5.39 (1H, dd, *J* = 9.7, 7.9 Hz, H-2Glc), 5.69 (1H, t, *J* = 9.7 Hz, H-4Glc'), 5.75 (1H, d, *J* = 3.9 Hz, H-1Glc'), 5.81 (1H, t, *J* = 9.4 Hz, H-3Glc), 6.11 (1H, t, *J* = 9.9 Hz, H-3Glc'). ^{13}C-NMR ($CDCl_3$) δ: 62.6 (C-6Glc), 63.5 (C-6Glc'), 69.1 (C-4Glc), 69.2 (C-5Glc'), 69.9 (C-3Glc'), 70.7 (C-2Glc'), 72.4 (C-5Glc), 72.5 (C-2Glc), 74.2 (C-4Glc), 74.9 (C-3Glc), 96.6 (C-1Glc'), 102.2 (C-1Glc). ESI-MS *m/z* : 1670 $[M+2H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.34; H, 6.42.

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl]-23-*O*-benzoylhederagenate (17). This compound was prepared in 75% yield using the general glycosylation method described above. R_f = 0.48 (toluene/EtOAc, 9:1). $[\alpha]_D$: +98.5 (*c*=0.8, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) δ: 0.66 (3H, s, H-24), 0.71 (3H, s, H-26), 0.90 (s3H,, H-25), 0.94 (3H, s, H-29), 0.97 (3H, s, H-30), 1.01 (3H, s, H-27), 2.88 (1H, dd, *J* = 13.8, 3.9 Hz, H-18), 3.66 (1H, m, H-6Glc), 3.72 (1H, dd, *J* = 11.3, 4.5 Hz, H-3), 3.92 (1H, d, *J* = 11.5 Hz, H-23), 3.97 (1H, d, *J* = 11.5 Hz, H-23), 4.01 (1H, m, H-5Glc), 4.07 (1H, dd, *J* = 10.6, 6.6 Hz, H-6Glc), 4.43 (1H, dd, *J* = 11.4, 5.6 Hz, H-6Gal), 4.57 (3H, m, H-6Gal, C*H*$_2$CH=CH$_2$), 4.75 (1H, t, *J* = 6.4 Hz, H-5Gal), 4.92 (1H, d, *J* = 7.9 Hz, H-1Glc), 5.24 (1H, m, H-12), 5.43 (1H, dd, *J* = 9.9, 8.0 Hz, H-2Glc), 5.47 (2H, m, H-1Gal, H-4Glc), 5.77 (1H, dd, *J* = 10.6, 3.5 Hz, H-2Gal), 5.88 (1H, t, *J* = 9.7 Hz, H-3Glc), 6.07 (1H, m, H-4Gal), 6.12 (1H, dd, *J* = 10.6, 3.4 Hz, H-3Gal). ^{13}C-NMR ($CDCl_3$) δ: 62.5 (C-6Gal), 66.4 (C-6Glc), 67.3 (C-5Gal), 68.2 (C-3Gal), 69.2 (C-2Gal, C-4Gal), 69.4 (C-4Glc), 72.0 (C-2Glc), 72.8 (C-5Glc), 72.9 (C-3Glc), 96.5 (C-1Gal), 102.6 (C-1Glc). ESI-MS *m/z* : 1669 $[M+H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.52; H, 6.69.

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-*β*-D-glucopyranosyl]-23-*O*-benzoylhederagenate (18). This compound was prepared in 74% yield using the general glycosylation method described above. R_f = 0.52 (toluene/EtOAc, 9:1). $[\alpha]_D$: +79.5 (*c*=0.6, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) *δ*: 0.70 (3H, s, H-24), 0.73 (3H, s, H-26), 0.89 (3H, s, H-25), 0.94 (3H, s, H-29), 0.97 (3H, s, H-30), 1.02 (3H, s, H-27), 2.91 (1H, dd, *J* = 13.8, 4.1 Hz, H-18), 3.67 (1H, dd, *J* = 11.7, 4.5 Hz, H-3), 3.88 (1H, d, *J* = 11.5 Hz, H-23), 3.97 (1H, d, *J* = 11.4 Hz, H-23), 3.98 (1H, dd, *J* = 11.0, 6.7 Hz, H-6Glc), 4.07 (2H, m, H-6Glc, H-5Glc), 4.13 (1H, m, H-5Glc'), 4.43 (1H, dd, *J* = 12.2, 5.0 Hz, H-6Glc'), 4.55 (3H, m, H-6Glc', C*H*$_2$CH=CH$_2$), 4.90 (1H, d, *J* = 7.9 Hz, H-1Glc), 5.06 (1H, d, *J* = 7.8 Hz, H-1Glc'), 5.34 (1H, m, H-12), 5.39 (1H, t, *J* = 9.6 Hz, H-4Glc), 5.52 (2H, dd, *J* = 9.6, 7.9 Hz, H-2Glc, H-2Glc'), 5.63 (1H, t, *J* = 9.6 Hz, H-4Glc'), 5.83 (1H, t, *J* = 9.6 Hz, H-3Glc), 5.87 (1H, t, *J* = 9.5 Hz, H-3Glc'). ^{13}C-NMR ($CDCl_3$) *δ*: 63.0 (C-6Glc'), 67.9 (C-6Glc), 69.4 (C-4Glc'), 69.9 (C-4Glc), 71.7 (C-2Glc'), 72.0 (C-2Glc), 72.3 (C-5Glc'), 72.9 (C-3Glc', C-3Glc), 73.6 (C-5Glc), 100.6 (C-1Glc'), 102.6 (C-1Glc). ESI-MS *m/z* : 1669 $[M+H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.04; H, 6.42.

Allyl 3-*O*-[2,3,4,6-tetra-*O*-benzoyl-*α*-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-*β*-D-glucopyranosyl]-23-*O*-benzoylhederagenate (19). This compound was prepared in 67% yield using the general glycosylation method described above. R_f = 0.48 (toluene/EtOAc, 9:1). $[\alpha]_D$: +80.8 (*c*=0.6, $CHCl_3$). Selected NMR data: ^{1}H-NMR ($CDCl_3$) *δ*: 0.67 (3H, s, H-24), 0.68 (3H, s, H-26), 0.85 (3H, s, H-25), 0.95 (3H, s, H-29), 0.97 (3H, s, H-30), 1.03 (3H, s, H-27), 2.87 (1H, dd, *J* = 13.5, 3.9 Hz, H-18), 3.67 (2H, m, H-6Glc, H-3), 3.91 (1H, d, *J* = 11.4 Hz, H-23), 3.97 (1H, d, *J* = 11.5 Hz, H-23), 4.02 (1H, m, H-5Glc), 4.09 (1H, dd, *J* = 11.0, 5.9 Hz, H-6Glc), 4.43 (1H, dd, *J* = 12.0, 5.2 Hz, H-6Glc'), 4.50 (1H, m, H-5Glc'), 4.55 (3H, m, H-6Glc',

C*H*$_2$CH=CH$_2$), 4.94 (1H, d, *J* = 7.9 Hz, H-1Glc), 5.17 (1H, m, H-12), 5.41 (2H, m, H-2Glc', H-1Glc'), 5.46 (1H, dd, *J* = 9.4, 8.2 Hz, H-2Glc), 5.58 (1H, t, *J* = 9.7 Hz, H-4Glc), 5.73 (1H, t, *J* = 9.8 Hz, H-4Glc'), 5.88 (1H, t, *J* = 9.8 Hz, H-3Glc), 6.27 (1H, t, *J* = 9.4 Hz, H-3Glc'). ^{13}C-NMR ($CDCl_3$) δ: 62.7 (C-6Glc'), 66.8 (C-6Glc), 68.0 (C-4Glc'), 69.3 (C-4Glc', C-4Glc), 70.5 (C-3Glc'), 71.8 (C-2Glc'), 72.0 (C-2Glc), 72.8 (C-5Glc), 73.0 (C-3Glc), 96.4 (C-1Glc'), 102.8 (C-1Glc). ESI-MS *m/z* : 1669 $[M+H]^+$. *Anal.* Calcd for $C_{101}H_{104}O_{22}$: C, 72.65; H, 6.28. Found: C, 72.55; H, 6.15.

3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (1). General Method In a typical experiment saponin **14** (0.90 g, 0.53 mmol) was treated with a solution of 3% KOH in MeOH (56 ml). The reaction was stirred for 48h before being neutralized with Amberlite IR 120 (H^+ form), filtered and evaporated. The residue was then taken up in anhydrous THF (32 ml) and piperidine (0.16 ml, 1.6 mmol, 3 eq), tetrakistriphenylphosphine (0.184 g, 0.16 mmol, 0.3 eq), and triphenylphosphine (0.042 g, 0.16 mmol, 0.3 eq) were added. After 3 days, the solvent was evaporated and the residue purified by column chromatography (CH_2Cl_2/CH_3OH/H_2O, 82:18:0.5) to give 0.341 g (81%) of the saponin **1**. Spectral identification was performed in pyridine- d_5 and was in accordance with published data.[18)]

3-*O*-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (2). This compound was prepared in 71% yield using the general deprotection method described above. $[\alpha]_D$: +26.6 (*c*=0.5, pyridine). ^{1}H-NMR (pyridine d_5) δ: 0.89 (3H, s, H-25), 0.90 (3H, s, H-29), 0.94 (3H, s, H-24), 0.96-2.17 (22H, m, H-1, H-2, H-5, H-6, H-7, H-9, H-11, H-15, H-16, H-19, H-21, H-22), 0.98 (3H, s, H-30), 1.00 (3H, s, H-26), 1.24 (3H, s, H-27), 3.28 (1H, dd, *J* = 13.8, 3.8 Hz, H-18), 3.68 (1H, d, *J* = 11.0 Hz, H-23), 3.84 (1H, dt, *J* = 9.4, 3.2 Hz, H-5Glc), 4.03 (1H, t, *J* = 8.4 Hz, H-2Glc), 4.11 (1H, m, H-5Gal), 4.14 (1H, dd, *J* = 9,6, 3.3 Hz, H-3Gal), 4.20 (1H, dd, *J*

= 11.7, 3.0 Hz, H-3), 4.21 (1H, t, *J* = 8.8 Hz, H-3Glc), 4.29 (1H, t, *J* = 9.2 Hz, H-4Glc), 4.32 (1H, d, *J* = 10.9 Hz, H-23), 4.36 (1H, dd, *J* = 11.0, 5.3 Hz, H-6Gal), 4.43 (1H, dd, *J* = 10.9, 3.5 Hz, H-6Glc), 4.44 (1H, dd, *J* = 11.0, 7.2 Hz, H-6Gal), 4.47 (1H, brd, *J* = 3.1 Hz, H-4Gal), 4.51 (1H, dd, *J* = 9.3, 8.0 Hz, H-2Gal), 4.53 (1H, dd, *J* = 11.9, 3.8 Hz, H-6Glc), 5.09 (1H, d, *J* = 8.0 Hz, H-1Glc), 5.08 (1H, d, *J* = 7.9 Hz, H-1Gal), 5.46 (1H, brt, *J* = 3.1 Hz, H-12). ^{13}C-NMR (pyridine d_5) δ: 13.4 (C-24), 15.9 (C-25), 17.3 (C-26), 18.0 (C-6), 23.5 (C-16), 23.6 (C-30), 23.7 (C-11), 25.6 (C-2), 26.0 (C-27), 28.1 (C-15), 30.8 (C-20), 32.7 (C-7), 33.1 (C-22, C-29), 34.0 (C-21), 36.7 (C-10), 38.4 (C-1), 39.6 (C-8), 41.8 (C-18), 42.0 (C-14), 43.2 (C-4), 46.2 (C-19), 46.5 (C-17), 47.4 (C-5), 47.9 (C-9), 61.7 (C-6Gal), 61.8 (C-6Glc), 64.3 (C-23), 69.7 (C-4Gal), 72.2 (C-2Gal), 74.8 (C-3Gal), 75.0 (C-2Glc), 76.1 (C-5Glc), 76.5 (C-3Glc), 77.0 (C-5Gal), 81.8 (C-4Glc), 82.2 (C-3), 105.2 (C-1Glc), 105.6 (C-1Gal), 122.3 (C-12), 144.8 (C-13), 180.2 (C-28). ESI-MS *m/z* : 797 $[M+H]^+$. *Anal.* Calcd for $C_{42}H_{68}O_{14}$ (·4.2 H_2O) : C, 57.81; H, 8.82. Found: C, 57.82; H, 9.07.

3-*O*-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl]hederagenin (3). This compound was prepared in 78% yield using the general deprotection method described above. $[\alpha]_D$: +62.4 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (pyridine d_5) δ: 0.88 (3H, s, H-25), 0.91 (3H, s, H-29), 0.93 (3H, s, H-24), 0.98 (3H, s, H-30), 0.99 (3H, s, H-26), 1.24 (3H, s, H-27), 3.27 (1H, dd, *J* = 13.7, 3.7 Hz, H-18), 3.69 (1H, d, *J* = 11.0 Hz, H-23), 3.72 (1H, dt, *J* = 9.1, 2.6 Hz, H-5Glc), 4.01 (1H, t, *J* = 8.4 Hz, H-2Glc), 4.15 (1H, t, *J* = 9.1 Hz, H-4Glc'), 4.16 (1H, dd, *J* = 9.5, 3.9 Hz, H-2Glc'), 4.24 (1H, dd, *J* = 12.1, 4.4 Hz, H-3), 4.26 (1H, t, *J* = 8.6 Hz, H-3Glc), 4.31 (1H, t, *J* = 9.2 Hz, H-4Glc), 4.32 (2H, m, H-23, H-6Glc'), 4.42 (1H, m, H-6Glc), 4.45 (1H, m, H-6Glc), 4.55 (2H, m, H-5Glc', H-6Glc'), 4.58 (1H, t, *J* = 9.2 Hz, H-3Glc'), 5.06 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.48 (1H, m, H-12), 5.88 (1H, d, *J* = 3.8 Hz, H-1Glc'). ^{13}C-NMR (pyridine d_5) δ: 61.5 (C-6Glc), 62.3 (C-6Glc'), 71.4 (C-4Glc'), 74.0 (C-2Glc'), 74.8 (C-2Glc),

74.9 (C-5Glc'), 75.0 (C-3Glc'), 76.2 (C-5Glc), 77.6 (C-3Glc), 80.9 (C-4Glc), 102.7 (C-1Glc'), 105.3 (C-1Glc). ESI-MS *m/z* : 797 $[M+H]^+$.

3-*O*-[α-D-galactopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (4). This compound was prepared in 75% yield using the general deprotection method described above. $[\alpha]_D$: +55.6 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (pyridine d_5) δ: 0.87 (3H, s, H-25), 0.90 (3H, s, H-24), 0.90 (3H, s, H-29), 0.98 (6H, s, H-26, H-30), 1.20 (3H, s, H-27), 3.26 (1H, dd, *J* = 13.5, 3.5 Hz, H-18), 3.66 (1H, d, *J* = 10.8 Hz, H-23), 3.97 (1H, ddd, *J* = 8.2, 6.1, 1.6 Hz, H-5Glc), 4.03 (1H, t, *J* = 8.7 Hz, H-2Glc), 4.06 (1H, t, *J* = 9.4 Hz, H-4Glc), 4.12 (1H, t, *J* = 8.9 Hz, H-3Glc), 4.22 (1H, dd, *J* = 11.9, 4.3 Hz, H-3), 4.26 (1H, brd, *J* = 8.8 Hz, H-6Glc), 4.32 (1H, d, *J* = 11.0 Hz, H-23), 4.39 (2H, brd, *J* = 6.0 Hz, 2 × H-6Gal), 4.49 (1H, dd, *J* = 10.3, 6.2 Hz, H-6Glc), 4.56 (1H, dd, *J* = 9.9, 3.2 Hz, H-3Gal), 4.59 (1H, brd, *J* = 2.6 Hz, H-4Gal), 4.63 (1H, brd, *J* = 7.4 Hz, H-5Gal), 4.66 (1H, dd, *J* = 9.8, 3.6 Hz, H-2Gal), 5.06 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.40 (1H, m, H-12), 5.47 (1H, d, *J* = 3.5 Hz, H-1Gal). ^{13}C-NMR (pyridine d_5) δ: 62.1 (C-6Gal), 67.6 (C-6Glc), 70.3 (C-2Gal), 70.5 (C-4Gal), 71.1 (C-3Gal), 71.5 (C-4Glc), 72.2 (C-5Gal), 75.1 (C-2Glc), 75.7 (C-5Glc), 78.2 (C-3Glc), 100.1 (C-1Gal), 105.8 (C-1Glc). ESI-MS *m/z* : 797 $[M+H]^+$.

3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (5). This compound was prepared in 88% yield using the general deprotection method described above. $[\alpha]_D$: +13.4 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (pyridine d_5) δ: 0.88 (3H, s, H-25), 0.91 (3H, s, H-29), 0.94 (3H, s, H-24), 1.00 (3H, s, H-26), 1.02 (3H, s, H-30), 1.21 (3H, s, H-27), 3.39 (1H, dd, *J* = 13.8, 3.5 Hz, H-18), 3.67 (1H, d, *J* = 10.8 Hz, H-23), 3.90 (1H, m, H-5Glc'), 3.97 (1H, t, *J* = 8.3 Hz, H-2Glc), 4.00 (1H, m, H-5Glc), 4.04 (1H, t, *J* = 8.1 Hz, H-2Glc'), 4.13 (1H, t, *J* = 8.8 Hz, H-3Glc), 4.18 (1H, t, *J* = 9.2 Hz, H-4Glc), 4.22 (1H, m, H-4Glc'), 4.23 (2H, m,

H-3Glc', H-3), 4.32 (1H, dd, *J* = 11.7, 6.5 Hz, H-6Glc), 4.33 (1H, d, *J* = 10.8 Hz, H-23), 4.34 (1H, dd, *J* = 11.8, 5.2 Hz, H-6Glc'), 4.48 (1H, dd, *J* = 11.8, 2.2 Hz, H-6Glc'), 4.79 (1H, brd, *J* = 10.3 Hz, H-6Glc), 5.08 (1H, d, *J* = 7.7 Hz, H-1Glc'), 5.09 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.45 (1H, m, H-12). ^{13}C-NMR (pyridine d_5) δ: 62.3 (C-6Glc'), 70.0 (C-6Glc), 71.1 (C-4Glc), 71.2 (C-4Glc'), 74.8 (C-2Glc'), 75.3 (C-2Glc), 76.6 (C-5Glc), 78.0 (C-3Glc'), 78.1 (C-3Glc), 78.2 (C-5Glc'), 105.1 (C-1Glc'), 105.8 (C-1Glc). ESI-MS *m/z* : 797 $[M+H]^+$. *Anal.* Calcd for $C_{42}H_{68}O_{14}$ (·4.2 CH_3OH) : C, 59.57; H, 9.17. Found: C, 59.55; H, 9.18.

3-*O*-[α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]hederagenin (6). This compound was prepared in 74% yield using the general deprotection method described above. $[\alpha]_D$: +54.8 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (pyridine d_5) δ: 0.90 (3H, s, H-25), 0.92 (3H, s, H-29), 0.93 (3H, s, H-24), 0.99 (6H, s, H-26, H-30), 1.19 (3H, s, H-27), 3.29 (1H, dd, *J* = 13.8, 3.9 Hz, H-18), 3.68 (1H, d, *J* = 10.8 Hz, H-23), 3.97 (1H, ddd, *J* = 9.2, 5.9, 2.2 Hz, H-5Glc), 4.00 (1H, t, *J* = 8.3 Hz, H-2Glc), 4.06 (1H, dd, *J* = 9.2, 8.7 Hz, H-4Glc), 4.11 (1H, t, *J* = 8.7 Hz, H-3Glc), 4.14 (1H, dd, *J* = 9.6, 3.7 Hz, H-2Glc'), 4.23 (1H, t, *J* = 9.3 Hz, H-4Glc'), 4.27 (1H, dd, *J* = 11.9, 4.5 Hz, H-3), 4.33 (1H, dd, *J* = 10.9, 1.8 Hz, H-6Glc), 4.35 (1H, d, *J* = 10.8 Hz, H-23), 4.36 (1H, dd, *J* = 11.7, 4.5 Hz, H-6Glc'), 4.46 (1H, dd, *J* = 11.7, 2.4 Hz, H-6Glc'), 4.51 (1H, dd, *J* = 10.9, 5.8 Hz, H-6Glc), 4.54 (1H, ddd, *J* = 9.9, 4.9, 2.4 Hz, H-5Glc'), 4.62 (1H, t, *J* = 9.2 Hz, H-3Glc'), 5.08 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.39 (1H, brt, *J* = 3.1 Hz, H-12), 5.47 (1H, d, *J* = 3.7 Hz, H-1Glc'). ^{13}C-NMR (pyridine d_5) δ: 62.3 (C-6Glc'), 68.3 (C-6Glc), 71.6 (C-4Glc, C-4Glc'), 73.6 (C-5Glc', C-2Glc'), 74.9 (C-3Glc'), 75.1 (C-2Glc), 75.8 (C-5Glc), 78.2 (C-3Glc), 100.1 (C-1Glc'), 105.8 (C-1Glc). ESI-MS *m/z* : 797 $[M+H]^+$.

The saponin methyl esters were prepared using diazomethane[26] in quantitative yields.

Methyl 3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]hederagenate (1a). $[\alpha]_D$: +28.0 (*c*=0.5, pyridine). ^{1}H-NMR (CD_3OD) *δ*: 0.73 (3H, s, H-24), 0.77 (3H, s, H-26), 0.93 (3H, s, H-29), 0.96 (3H, s, H-30), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.8, 4.0 Hz, H-18), 3.25 (1H, dd, *J* = 9.0, 7.9 Hz, H-2Glc'), 3.26 (1H, dd, *J* = 9.0, 7.6 Hz, H-2Glc), 3.30 (1H, d, *J* = 11.4 Hz, H-23), 3.32 (1H, t, *J* = 9.3 Hz, H-4Glc'), 3.38 (1H, ddd, *J* = 9.3, 5.7, 2.1 Hz, H-5Glc'), 3.39 (1H, t, *J* = 9.1 Hz, H-3Glc'), 3.42 (1H, dt, *J* = 9.6, 2.8 Hz, H-5Glc), 3.52 (1H, t, *J* = 9.0 Hz, H-3Glc), 3.59 (1H, t, *J* = 9.0 Hz, H-4Glc), 3.64 (3H, s, OC*H*$_3$), 3.64 (1H, m, H-3), 3.65 (1H, d, *J* = 11.4 Hz, H-23), 3.68 (1H, dd, *J* = 12.0, 5.7 Hz, H-6Glc'), 3.87 (1H, dd, *J* = 12.1, 2.6 Hz, H-6Glc), 3.90 (1H, m, H-6Glc), 3.90 (1H, dd, *J* = 12.0, 2.0 Hz, H-6Glc'), 4.44 (1H, d, *J* = 7.9 Hz, 1H, H-1Glc'), 4.45 (1H, d, *J* = 7.9 Hz, H-1Glc), 5.27 (1H, brt, *J* = 3.2 Hz, H-12). ^{13}C-NMR (CD_3OD) *δ*: 11.9 (C-24), 14.9 (C-25), 16.2 (C-26), 17.4 (C-6), 22.5 (C-30), 22.6 (C-16), 23.1 (C-11), 24.9 (C-2), 25.0 (C-27), 27.3 (C-15), 30.1 (C-20), 31.9 (C-7), 32.0 (C-29), 32.1 (C-22), 33.3 (C-21), 36.2 (C-10), 38.0 (C-1), 39.1 (C-8), 41.3 (C-18), 41.4 (C-14), 42.4 (C-4), 45.6 (C-19), 46.6 (C-17), 46.7 (C-5), 47.4 (C-9), 50.7 (O*C*H$_3$), 60.4 (C-6Glc), 61.0 (C-6Glc'), 63.3 (C-23), 69.9 (C-4Glc'), 73.5 (C-2Glc'), 73.8 (C-2Glc), 74.8 (C-5Glc), 75.2 (C-3Glc), 76.4 (C-3Glc'), 76.6 (C-5Glc'), 79.2 (C-4Glc), 81.9 (C-3), 103.1 (C-1Glc'), 104.1 (C-1Glc), 122.3 (C-12), 143.6 (C-13), 178.6 (C-28). ESI-MS *m/z* : 811 $[M+H]^+$.

Methyl 3-*O*-[*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranosyl]hederagenate (2a). $[\alpha]_D$: +29.4 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (CD_3OD) *δ*: 0.73 (3H, s, H-24), 0.77 (3H, s, H-26), 0.93 (3H, s, H-29), 0.96 (3H, s, H-30), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.4, 4.0 Hz, H-18), 3.26 (1H, dd, *J* = 9.0, 8.0 Hz, H-2Glc), 3.30 (1H, d, *J* = 11.4 Hz, H-23), 3.42 (1H, dt, *J* = 9.5, 3.0 Hz, H-5Glc), 3.50 (1H, dd, *J* = 9.8, 3.2 Hz, H-3Gal), 3.53 (1H, t, *J* = 9.3 Hz, H-3Glc), 3.56 (1H, dd, *J* = 9.6, 7.6 Hz, H-2Gal), 3.59 (1H, t, *J* = 9.3 Hz, H-4Glc), 3.60 (1H, ddd, *J* = 4.2, 3.2, 1.0 Hz, H-5Gal), 3.64 (3H, s, OC*H*$_3$), 3.66 (2H, m, H-3, H-23), 3.72 (1H, dd, *J* = 11.5, 4.6 Hz, H-6Gal), 3.80 (1H, dd, *J* = 11.5, 7.5 Hz, H-6Gal), 3.83

(1H, brd, *J* = 2.8 Hz, H-4Gal), 3.88 (2H, brd, *J* = 3.0 Hz, H-6Glc, H-6Glc), 4.39 (1H, d, *J* = 7.6 Hz, H-1Gal), 4.45 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.27 (1H, brt, *J* = 3.3 Hz, H-12). ^{13}C-NMR (CD_3OD) *δ*: 50.7 (OCH_3), 60.5 (C-6Glc), 61.0 (C-6Gal), 68.9 (C-4Gal), 71.1 (C-2Gal), 73.4 (C-3Gal), 73.8 (C-2Glc), 74.8 (C-5Gal), 75.2 (C-3Glc), 75.6 (C-5Glc), 79.1 (C-4Glc), 103.6 (C-1Gal), 104.1 (C-1Glc). ESI-MS *m/z* : 811 $[M+H]^+$. *Anal.* Calcd for $C_{43}H_{70}O_{14}$ (·3.5 CH_3OH) : C, 60.50; H, 9.17. Found: C, 60.54; H, 9.33.

Methyl 3-*O*-[*α*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl]hederagenate (3a). $[\alpha]_D$: +67.4 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (CD_3OD) *δ*: 0.73 (3H, s, H-24), 0.77 (3H, s, H-26), 0.93 (3H, s, H-29), 0.96 (3H, s, H-30), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.6, 3.6 Hz, H-18), 3.25 (1H, dd, *J* = 8.6, 8.2 Hz, H-2Glc), 3.28 (1H, t, *J* = 9.0 Hz, H-4Glc'), 3.30 (1H, d, *J* = 11.4 Hz, H-23), 3.39 (1H, m, H-5Glc), 3.47 (1H, dd, *J* = 9.7, 3.6 Hz, H-2Glc'), 3.57 (1H, t, *J* = 9.2 Hz, H-4Glc), 3.61 (1H, m, H-3Glc), 3.62 (3H, s, OCH_3), 3.62 (1H, m, H-3Glc'), 3.63 (1H, d, *J* = 11.4 Hz, H-23), 3.66 (1H, m, H-3), 3.68 (1H, m, H-6Glc'), 3.72 (1H, m, H-5Glc'), 3.84 (2H, m, H-6Glc, H-6Glc'), 3.87 (1H, m, H-6Glc), 4.43 (1H, d, *J* = 7.8 Hz, H-1Glc), 5.19 (1H, d, *J* = 3.7 Hz, H-1Glc'), 5.27 (1H, m, H-12). ^{13}C-NMR (CD_3OD) *δ*: 50.8 (OCH_3), 60.7 (C-6Glc), 61.3 (C-6Glc'), 70.1 (C-4Glc'), 72.7 (C-2Glc'), 73.3 (C-5Glc'), 73.6 (C-3Glc'), 73.7 (C-2Glc), 75.0 (C-5Glc), 76.6 (C-3Glc), 79.7 (C-4Glc), 101.4 (C-1Glc'), 104.2 (C-1Glc). ESI-MS *m/z* : 811 $[M+H]^+$. *Anal.* Calcd for $C_{43}H_{70}O_{14}$ (·2.5 H_2O) : C, 60.33; H, 8.83. Found: C, 60.37; H, 8.69.

Methyl 3-*O*-[*α*-D-galactopyranosyl-(1→6)-*β*-D-glucopyranosyl]hederagenate (4a). $[\alpha]_D$: +27.4 (*c*=0.5, pyridine). Selected NMR data: ^{1}H-NMR (CD_3OD) δ: 0.74 (3H, s, H-24), 0.77 (3H, s, H-26), 0.93 (3H, s, H-29), 0.96 (3H, s, H-30), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.9, 4.5 Hz, H-18), 3.21 (1H, t, *J* = 8.4 Hz, H-2Glc), 3.30 (1H, d, *J* = 11.3 Hz, H-23), 3.33 (1H, t, *J* = 9.4 Hz, H-4Glc), 3.37 (1H, t, *J* = 8.9 Hz, H-3Glc), 3.53 (1H, ddd, *J*

= 9.4, 5.8, 2.1 Hz, H-5Glc), 3.64 (3H, s, OC*H*$_3$), 3.66 (1H, d, *J* = 11.3 Hz, H-23; 1H, dd, *J* = 11.9, 3.3 Hz, H-3), 3.71 (1H, dd, *J* = 10.5, 2.1 Hz, H-6Glc), 3.73 (2H, dd, *J* = 11.4, 2.6 Hz, 2 × H-6Gal), 3.77 (2H, m, H-2Gal, H-3Gal), 3.90 (1H, m, H-4Gal), 3.91 (1H, dd, *J* = 10.1, 6.0 Hz, H-6Glc; 1H, m, H-5Gal), 4.44 (1H, d, *J* = 7.9 Hz, H-1Glc), 4.93 (1H, m, H-1Gal), 5.27 (1H, brt, *J* = 3.6 Hz, H-12). ^{13}C-NMR (CD_3OD) *δ*: 50.7 (O*C*H$_3$), 61.2 (C-6Gal), 66.2 (C-6Glc), 69.1 (C-2Gal), 69.6 (C-4Gal), 70.2 (C-3Gal), 70.3 (C-4Glc), 70.8 (C-5Gal), 74.2 (C-2Glc), 74.7 (C-5Glc), 76.9 (C-3Glc), 98.6 (C-1Gal), 104.5 (C-1Glc). ESI-MS *m/z* : 811 $[M+H]^+$.

Methyl 3-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]hederagenate (5a). $[\alpha]_D$: +8.2 (*c*=0.5, CH_3OH). Selected NMR data: ^{1}H-NMR (CD_3OD) *δ*: 0.73 (3H, s, H-24), 0.77 (3H, s, H-26), 0.93 (3H, s, H-29), 0.96 (3H, s, H-30), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.7, 4.3 Hz, H-18), 3.20 (1H, dd, *J* = 9.2, 7.8 Hz, H-2Glc), 3.23 (1H, dd, *J* = 9.1, 7.8 Hz, H-2Glc'), 3.29 (1H, m, H-5Glc'), 3.30 (1H, d, *J* = 11.8 Hz, H-23), 3.31 (1H, t, *J* = 8.7 Hz, H-4Glc'), 3.35 (1H, m, H-4Glc), 3.36 (1H, m, H-3Glc), 3.38 (1H, t, *J* = 8.7 Hz, H-3Glc'), 3.48 (1H, ddd, *J* = 9.8, 5.5, 1.8 Hz, H-5Glc), 3.64 (3H, s, OC*H*$_3$), 3.66 (2H, m, H-3, H-23), 3.69 (1H, dd, *J* = 12.0, 5.4 Hz, H-6Glc'), 3.81 (1H, dd, *J* = 11.7, 5.5 Hz, H-6Glc), 3.91 (1H, dd, *J* = 12.0, 2.1 Hz, H-6Glc'), 4.13 (1H, dd, *J* = 11.7, 2.0 Hz, H-6Glc), 4.40 (1H, d, *J* = 7.8 Hz, H-1Glc'), 4.43 (1H, d, *J* = 7.9 Hz, H-1Glc), 5.27 (1H, brt, *J* = 3.4 Hz, H-12). ^{13}C-NMR (CD_3OD) *δ*: 50.7 (O*C*H$_3$), 61.3 (C-6Glc'), 68.4 (C-6Glc), 70.0 (C-4Glc), 70.1 (C-4Glc'), 73.7 (C-2Glc'), 74.1 (C-2Glc), 75.4 (C-5Glc), 76.5 (C-3Glc', C-5Glc'), 76.7 (C-3Glc), 103.3 (C-1Glc'), 104.3 (C-1Glc). ESI-MS *m/z* : 811 $[M+H]^+$. *Anal.* Calcd for $C_{43}H_{70}O_{14}$ (·3.4 H_2O) : C, 59.21; H, 8.87. Found: C, 59.20; H, 9.11.

Methyl 3-*O*-[*α*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl]hederagenate (6a). $[\alpha]_D$: +46.3 (*c*=1.0, CH_3OH). Selected NMR data: ^{1}H-NMR (CD_3OD) *δ*: 0.74 (3H, s, H-24), 0.77

(3H, s, H-26), 0.93 (3H, s, H-30), 0.96 (3H, s, H-29), 1.00 (3H, s, H-25), 1.19 (3H, s, H-27), 2.89 (1H, dd, *J* = 13.7, 3.8 Hz, H-18), 3.21 (1H, t, *J* = 8.4 Hz, H-2Glc), 3.30 (1H, d, *J* = 11.8 Hz, H-23), 3.34 (1H, m, H-4Glc'), 3.37 (3H, m, H-3Glc, H-4Glc, H-2Glc'), 3.51 (1H, m, H-5Glc), 3.65 (3H, s, OC*H*$_3$), 3.65-3.73 (5H, m, H-3, H-3Glc', H-5Glc', H-6Glc', H-6Glc), 3.67 (1H, d, *J* = 11.7 Hz, H-23), 3.82 (1H, brd, *J* = 9.6 Hz, H-6Glc'), 3.96 (1H, dd, *J* = 10.8, 5.0 Hz, H-6Glc), 4.45 (1H, d, *J* = 7.9 Hz, H-1Glc), 4.87 (1H, d, *J* = 3.6 Hz, H-1Glc'), 5.27 (1H, m, H-12). ^{13}C-NMR (CD_3OD) δ: 50.7 (O*C*H$_3$), 61.1 (C-6Glc'), 66.3 (C-6Glc), 70.0 (C-4Glc), 70.2 (C-4Glc'), 72.0 (C-5Glc'), 72.4 (C-2Glc'), 73.8 (C-3Glc'), 74.1 (C-2Glc), 74.8 (C-5Glc), 76.9 (C-3Glc), 98.6 (C-1Glc'), 104.6 (C-Glc). ESI-MS *m/z* : 811 $[M+H]^+$.

Hemolytic tests. Sheep erythrocytes were purchased from Eurobio® with a determined hematocrite level. This suspension was diluted with phosphate buffer saline (PBS) (pH 7.4) in order to obtain a 10% solution. Mother saponin solutions (5.10^{-1} mg/mL) were freshly prepared by dissolving saponin in DMSO, then adding PBS to obtain a solution of 5:1 DMSO/PBS; 1 ml samples were prepared with concentrations ranging from 10^{-3} to 10^{-1} mg/mL (no damage of the erythrocytes caused by the DMSO occurred at 10^{-1} mg/mL). The erythrocyte suspension (25 μl) was added to 1 ml of the solution to be tested and the samples were rapidly stirred and incubated at 37°C with periodic stirring during the 60 min. incubation period. The solutions were then centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was measured at 540 nm and the hemolysis % was calculated by comparison with the 100% hemolysis caused by the Sigma® dialyzed mixture.

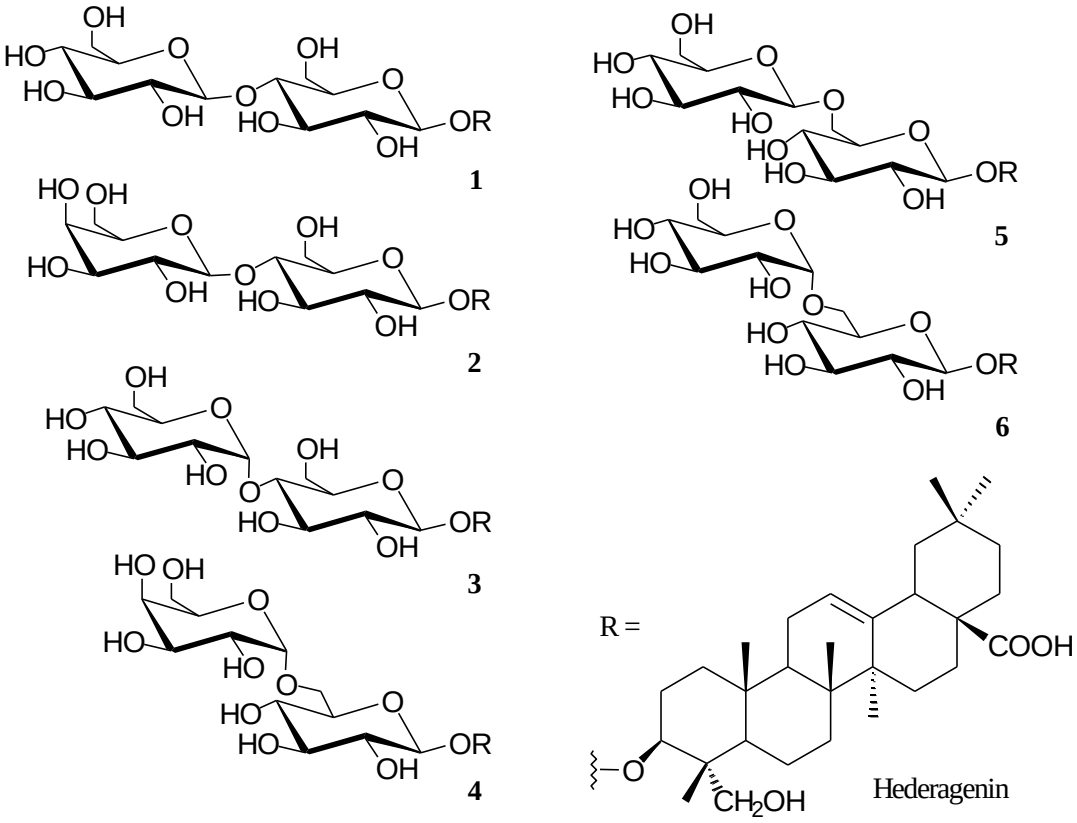
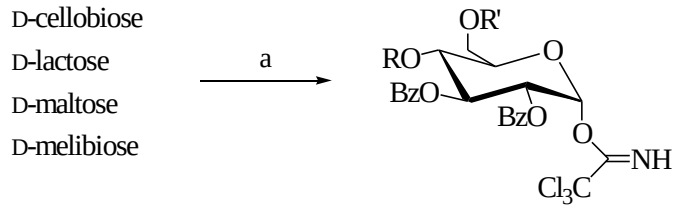


Chart 1



(7) R = 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl, R' = Bz (cellobiosyl)

(8) R = 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl, R' = Bz (lactosyl)

(9) R = 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl, R' = Bz (maltosyl)

(10) R = Bz, R' = 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl (melibiosyl)

Chart 2. Reagents and reactions conditions: (a) i) BzCl, Pyr; ii) HBr(33%)/HOAc, CH_2Cl_2; iii) Ag_2CO_3, Acetone/H_2O (1:1); iii) CCl_3CN, DBU, CH_2Cl_2, **7**, 69%; **8**, 63%; **9**, 86%; **10**, 68% (4 steps).

Chart 2

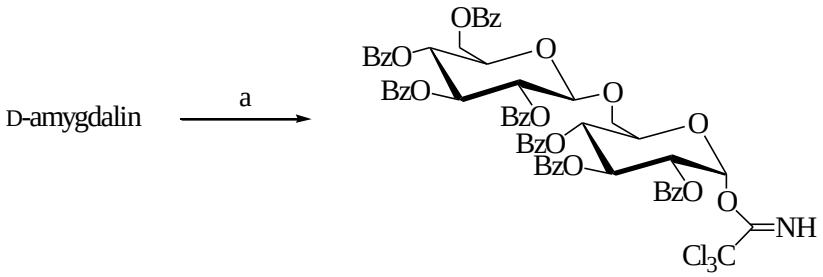
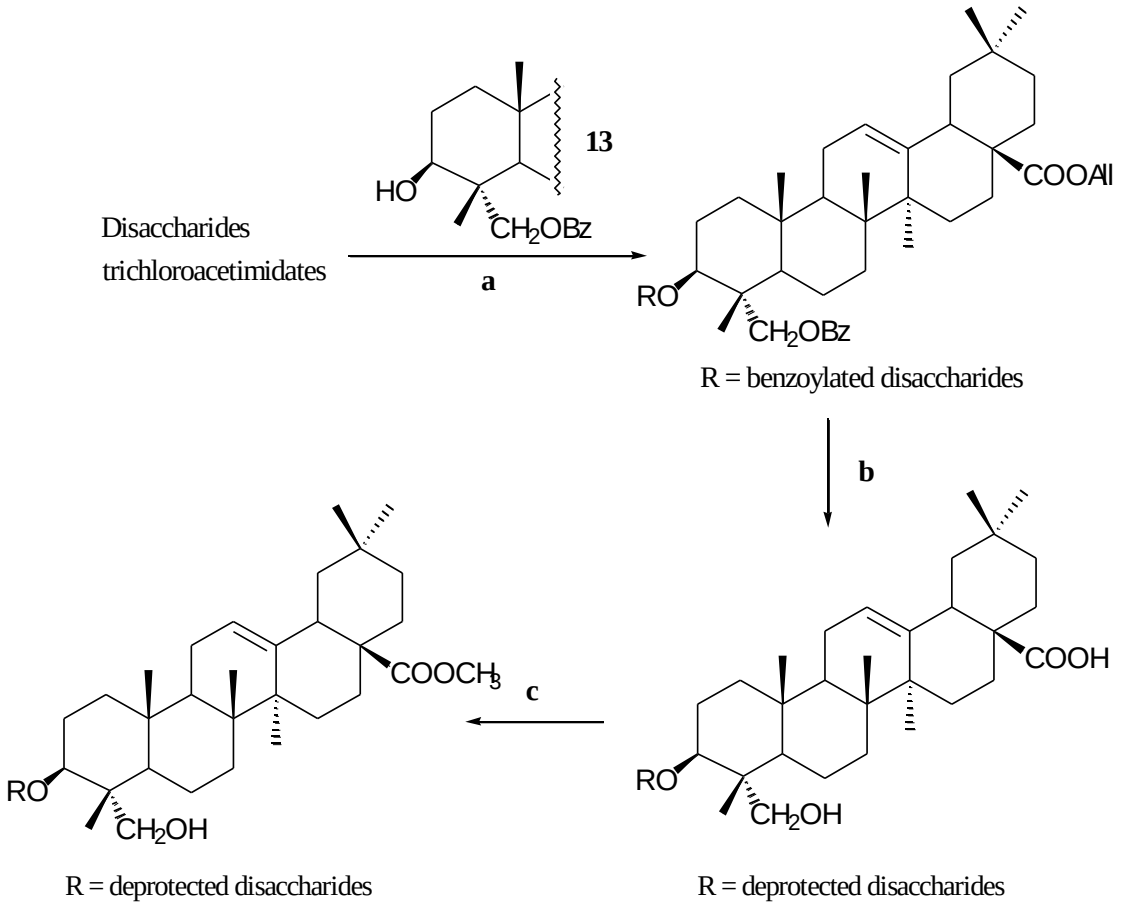


Chart 3. Reagents and reactions conditions: (a) i) BzCl, Pyr; ii) NH_4CO_2H, Pd/C, acetone; iii) CCl_3CN, DBU, CH_2Cl_2, **11,** 60% (3 steps).

Chart 3



Disaccharides trichloroacetimidates	**a**	Protected saponins	**b**	Deprotected saponins	**c**	Methyl ester saponins
7 (cellobiosyl)		**14** (70%)		**1** (81%)		**1a** (quantitative)
8 (lactosyl)		**15** (71%)		**2** (71%)		**2a** (quantitative)
9 (maltosyl)		**16** (70%)		**3** (78%)		**3a** (quantitative)
10 (melibiosyl)		**17** (75%)		**4** (75%)		**4a** (quantitative)
11 (gentiobiosyl)		**18** (74%)		**5** (88%)		**5a** (quantitative)
12 (isomaltosyl)		**19** (67%)		**6** (74%)		**6a** (quantitative)

Chart 4. Reagents and reactions conditions : (a) TMSOTf (0,05 eq), 4 Å molecular sieves, CH_2Cl_2, -20 °C; (b) i) 3% KOH/MeOH; ii) $Pd(PPh_3)_4$, piperidine, PPh_3, THF, 3d; c) CH_2N_2, Et_2O/MeOH.

Chart 4

Table 1 : Hemolytic activity of saponins (**1**-**6**) and their methyl ester (**1a**-**6a**) expressed as HD_{50} and HD_{100} (hemolytic dose for 50% and 100% hemolysis) in $\mu g.mL^{-1}$ as compared to dialyzed Sigma®.

Saponin	HD_{50}	HD_{100}	Saponin	HD_{50}	HD_{100}
1 (cellobiosyl)	22	30	**1a**	9	20
2 (lactosyl)	24	40	**2a**	7	10
3 (maltosyl)	41	100	**3a**	50	> 100 [65%]
4 (melibiosyl)	nha[a]	nha[a]	**4a**	42	> 100 [95%]
5 (gentiobiosyl)	nha[a]	nha[a]	**5a**	78	> 100 [80%]
6 (isomaltosyl)	nha[a]	nha[a]	**6a**	nha[a]	> 100 [6%]
Sigma®	9	30			

[a] nha : no hemolytic activity.

Table 1

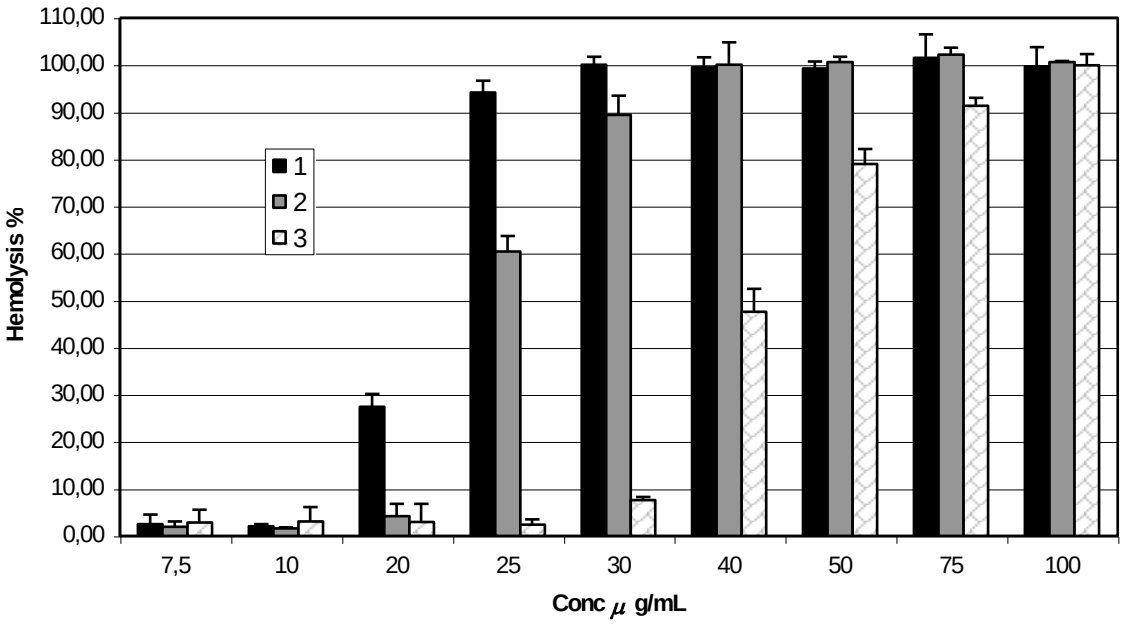


Figure 1 Hemolytic activity of saponins **1**-**3.**

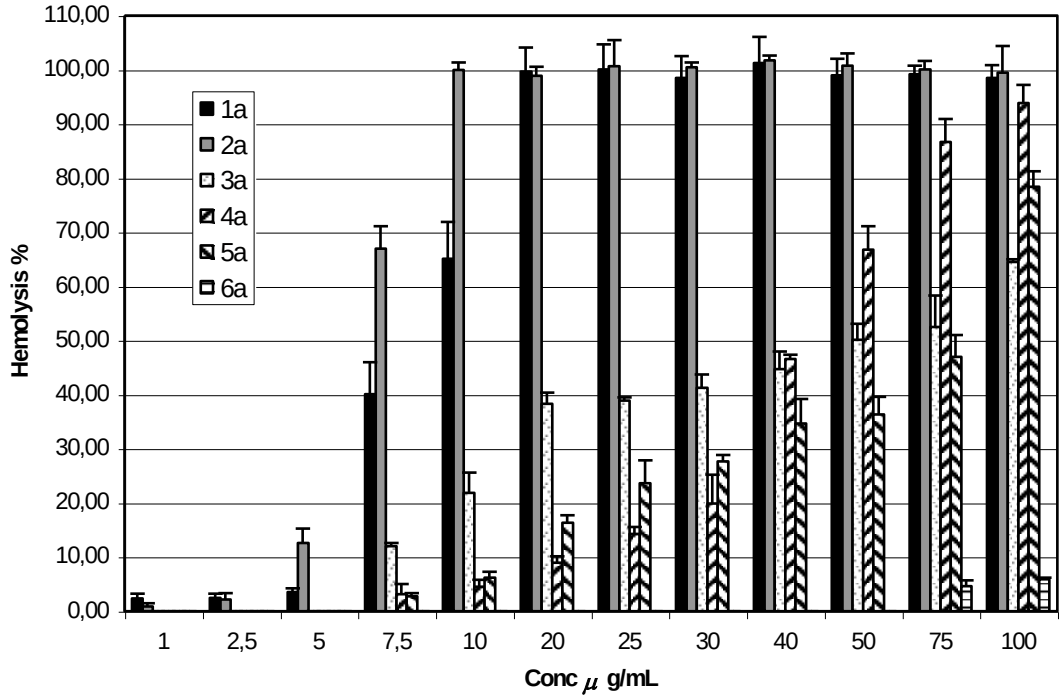


Figure 2 Hemolytic activity of methyl ester saponins **1a**-**6a**.

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