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Synthesis of flaccidoside II, a bidesmosidic triterpene saponin isolated from Chinese folk medicine Di Wu

Shuihong Cheng,[a] Yuguo Du,[a,*] Feihong Bing[b] and Guobin Zhang[b]

[a]*State Key Lab of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PR China*
[b]*Hubei College of Traditional Chinese Medicine, PR China*

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Abstract—A total synthesis of flaccidoside II, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, isolated from Chinese folk medicine Di Wu, has been accomplished from building blocks isopropyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-1-thio-β-D-xylopyranoside, 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate, oleanolic acid trityl ester, ethyl 2,3-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-glucopyranoside and 4-methoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside. The use of a partially protected thioglycosyl donor significantly simplified the synthesis of the target saponin.

Keywords: Flaccidoside II; Saponin; Di Wu; Natural product; Chinese folk medicine

1. Introduction

Since ancient times, traditional medicine has been used worldwide in the treatment of many diseases and for health care practices. Many compounds with well-defined biological and pharmacological activities have been isolated and structurally characterized from known medicinal plants.[1,2] Among these compounds, steroid or triterpene saponins present a broad spectrum of biomedical, food and industrial applications, which take advantages of their generally nonionic surfactant and membrane-disrupting properties. The useful commercial applications range from their use as fish and snail poisons, fire extinguishers, denatured alcohol to precursors for cortisone and other steroid drugs and hormones. Regarding the pharmaceutical uses, various saponins have shown interesting anti-cancer, anti-inflammatory, ion channel-blocking, immune-stimulating, antifungal, antithrombotic and hypocholesterolemic properties.[3–5]

Di Wu is the dry rhizome of *Anemone flaccida* Fr. Schmidt.[6] It is distributed in the southern part of China and is used as a folk medicine for detoxication, expelling wind-evil and releasing wetness-evil. The main bioactive fraction of Di Wu is proved to be triterpenoid saponins by pharmacological studies, and a bioactive component, named as flaccidoside II, was isolated from the alcohol extracts of *A. flaccida* Fr. Schmidt. On the basis of its chemical–physical properties, hydrolysis reactions and spectroscopic analyses, the chemical structure of flaccidoside II was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.[7] The structural complexity, especially having a 2′-OH branched sugar chain on C-3 of oleanolic acid, has hindered the chemical synthesis of this type of bidesmosidic triterpene saponin.[8] In the preparation of bioactive saponins containing a 2-OH branched sugar chain, we have recently developed a facile method by using partially protected glycosyl donors.[9] Herein, we report the synthesis of flaccidoside II by applying the same methodology.

* Corresponding author. Tel.: +86 10 62849126; fax: +86 10 62923563; e-mail: duyuguo@rcees.ac.cn

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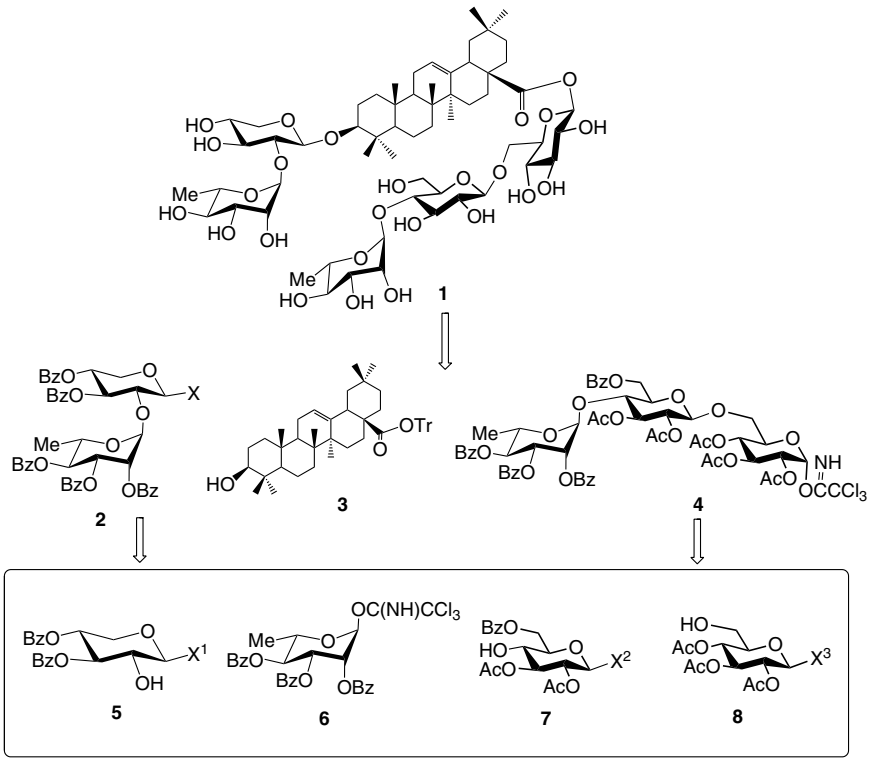
2. Results and discussion

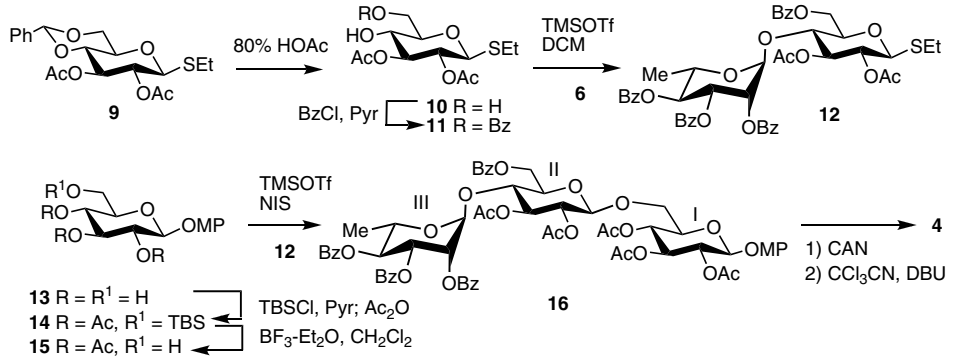
Saponin **1** can be disconnected into a disaccharide donor **2**, an oleanolic acid derivative **3** and a trisaccharide donor **4**. The sugar moieties **2** and **4** could be assembled from monosaccharide building blocks **5**, **6**, **7** and/or **8** through standard glycosylation procedures. As shown in Scheme 1, X, X^1, X^2 and X^3 represent suitable leaving groups or temporary protecting groups. The trityl ester **3** was easily prepared in quantitative yield by treating oleanolic acid with triphenylmethyl chloride (TrCl) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in refluxing tetrahydrofuran (THF), while 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**6**) is a known compound.[10]

As depicted in Scheme 2, trisaccharide trichloroacetimidate donor **4** was obtained in a straightforward manner. Thus, ethyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**9**)[11] was treated with aqueous 80% HOAc under reflux, giving ethyl 2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside (**10**), which was then regioselectively benzoylated with benzoyl chloride in pyridine at 0 °C obtained ethyl 2,3-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-glucopyranoside (**11**) in 85% yield from **9**. In the ^{1}H NMR spectrum of **11**, a set of doublet of doublet peaks at δ 4.61 and 4.77 ppm corresponding to H-6s clearly proved the selectivity. Coupling of L-rhamnopyranosyl trichloroacetimidate **6** and **11** in dry CH_2Cl_2 in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at 0 °C afforded ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-glucopyranoside (**12**) in 92% yield. Convergently, 4-methoxyphenyl β-D-glucopyranoside (**13**)[12] was regioselectively silylated with *tert*-butyldimethylsilyl chloride (TBSCl) in pyridine, followed by acetylation with acetic anhydride in one pot, to afford 4-methoxyphenyl 2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**14**) in good yield. $BF_3{\cdot}Et_2O$-catalyzed removal of TBS was carried out smoothly[13] to generate the acceptor, 4-methoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**15**) in excellent yield, with no acetyl migration[14] observed. Glycosylation of **12** and **15** in anhyd CH_2Cl_2 at ambient temperature using the *N*-iodosuccinimide (NIS)–TMSOTf combination successfully gave trisaccharide **16** in a yield of 86%. Treatment of **16** with ceric ammonium nitrate (CAN) in 4:1 CH_3CN–H_2O, followed by trichloroacetimidation (Cl_3CCN, DBU, CH_2Cl_2), furnished the trisaccharide building block **4** in 80% yield over two steps.

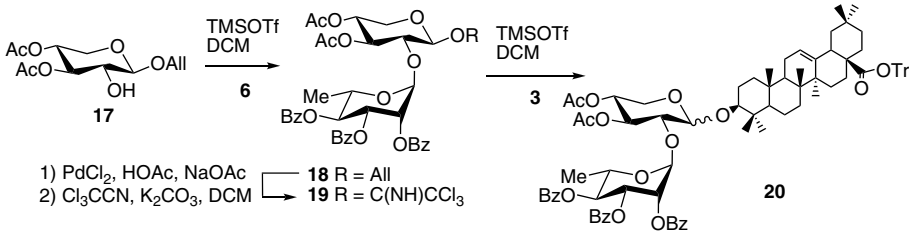
With compound **4** in hand, we started to formally assemble the target saponin **1**. The initial focus of this work was the synthesis of the disaccharide–oleanolic acid complex (Scheme 3). Thus, condensation of allyl 3,4-di-*O*-acetyl-β-D-xylopyranoside (**17**)[15] and **6** in dry CH_2Cl_2 with promotion of the reaction by TMSOTf generated disaccharide **18**, which was then transformed into a disaccharide donor **19** according to our published



Scheme 1. Retrosynthesis of Saponin **1**.



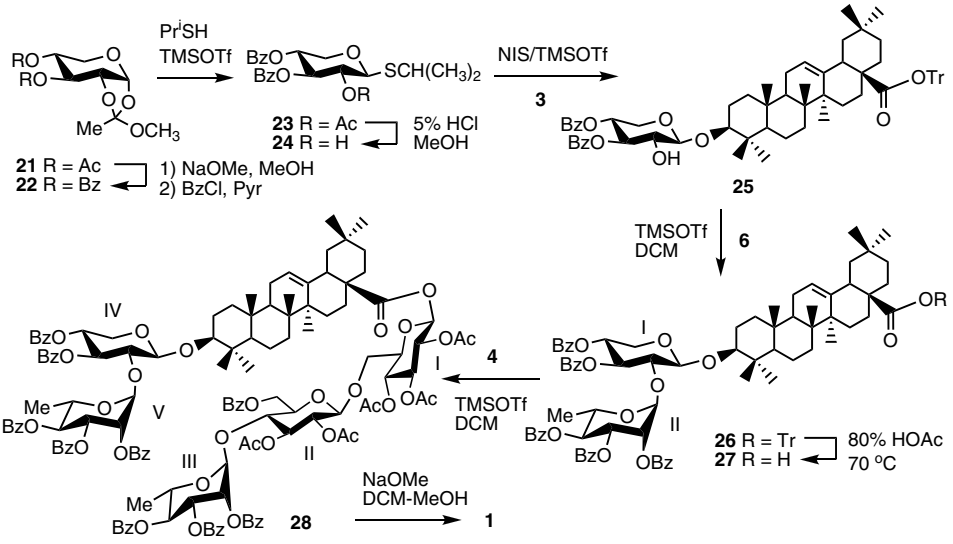
Scheme 2. Synthesis of trisaccharide **4**.



Scheme 3. Attempted strategy for the synthesis of disaccharide saponin derivative.

procedure ($PdCl_2$, HOAc, NaOAc; Cl_3CCN, K_2CO_3, CH_2Cl_2).[16] Coupling of oleanolic ester **3** with trichloroacetimidate **19** was completed within 30 min in the presence of a catalytic amount of TMSOTf at low temperature (−42 °C); however, an inseparable α,β-mixture of **20** was obtained. Limited efforts, owing to the instability of trityl ester, were tried regarding solvents (ether, toluene and acetonitrile) and catalysts (AgOTf and $HClO_4$–SiO_2),[17] but all were fruitless. We thus turned our attention to a stepwise route.

In our previous research, we found that a thioglycoside having its C-2 unprotected hydroxyl group could be a good glycosyl donor. Accordingly, orthoester **21**[18] was converted into 3,4-di-*O*-benzoyl-1,2-*O*-(1-methoxyethylidene)-α-D-xylopyranose (**22**), which was then dropped into a mixture of 2-PrSH and TMSOTf[19] in dry CH_2Cl_2 to afford isopropyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (**23**) (Scheme 4). Treatment of **23** with a methanolic solution containing 5% HCl (dry) gave the desired isopropyl 3,4-di-*O*-



Scheme 4. Final assembly of target compound **1**.

benzoyl-1-thio-β-D-xylopyranoside (**24**) in a moderate yield of 55% over two steps. Coupling of oleanolic ester **3** and thioglycoside **24** was carried out smoothly in dry dichloromethane at low temperature (−42 °C) under the promotion of co-catalyst NIS/TMSOTf, providing the desired saponin derivative **25** in 70% yield. Pure compound **25** was glycosylated with trichloroacetimidate **6** under standard conditions to give **26**, which was readily converted into acid **27** in aqueous 80% HOAc at 70 °C. Ester formation between acid **27** and trisaccharide donor **4** in dry dichloromethane under the promotion of TMSOTf led to the fully protected saponin derivative **28**. Selective removal of the acetate and benzoate protections using a catalytic amount of NaOMe (2:1 CH_2Cl_2–MeOH) in the presence of the C-28 ester glycosidic linkage finished the total synthesis of flaccidoside II. The physical data obtained were essentially identical to the natural product reported by us[6] and another group.[7] The prepared samples show good inhibition activity towards ConA-induced lymphocyte proliferation in a bioactivity screening,[20] and the details will be published in due course.

3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using a MALDITOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detection. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent, or a column of Bio-Gel P2 with water as the eluent. Solutions were concentrated at <50 °C under reduced pressure.

3.2. Ethyl 2,3-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-glucopyranoside (11)

Compound **9** (10.0 g, 25.2 mmol) was dissolved in 80% aq HOAc (100 mL). The mixture was stirred under reflux for 5 h and then co-evaporated with the help of toluene to dryness. The residue was subjected to column chromatography (1:1 EtOAc–petroleum ether) to give amorphous solid **10**, which was dissolved in pyridine (42 mL) and dropped into a mixture of benzoyl chloride (3.1 mL, 26.3 mmol) and pyridine (10 mL) at 0 °C. The mixture was stirred at rt for 6 h and then concentrated with toluene under reduced pressure. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give compound **11** as a foamy solid (8.83 g, 85% from **9**): $[\alpha]_D^{25}$ +65 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 2.09, 2.11 (2s, 2 × 3H, $2CH_3CO$), 2.66–2.78 (m, 2H, SCH_2CH_3), 3.68–3.69 (m, 2H, H-4, H-5), 4.55 (d, 1H, *J* 9.7 Hz, H-1), 4.61 (dd, 1H, *J* 1.3, 12.0 Hz, H-6a), 4.77 (dd, 1H, *J* 3.9, 12.0 Hz, H-6b), 5.01 (t, 1H, *J* 9.7 Hz, H-2), 5.13 (t, 1H, *J* 9.7 Hz, H-3), 7.47–8.10 (m, 5H, *Ph*). Anal. Calcd for $C_{19}H_{24}O_8S$: C, 55.33; H, 5.87. Found: C, 55.04; H, 5.72.

3.3. Ethyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzoyl-1-thio-β-D-glucopyranoside (12)

To a mixture of compounds **6** (1.10 g, 1.77 mmol) and **11** (610 mg, 1.48 mmol) in anhyd CH_2Cl_2 (10 mL) was added TMSOTf (32 μL, 0.17 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 30 min, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that all starting materials were consumed. The reaction mixture was neutralized with Et_3N and concentrated. Column chromatography (3:1 petroleum ether–EtOAc) of the residue gave **12** as a syrup (1.19 g, 92%): $[\alpha]_D^{25}$ +85 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 1.36 (d, 3H, *J* 6.3 Hz, H-6^{II}), 2.07, 2.09 (2s, 2 × 3H, $2CH_3CO$), 2.60–2.74 (m, 2H, SCH_2CH_3), 3.87–3.92 (m, 1H, H-5^I), 4.07 (t, 1H, *J* 9.3 Hz, H-4^I), 4.16–4.21 (m, 1H, H-5^{II}), 4.61 (d, 1H, *J* 10.2 Hz, H-1^I), 4.64 (dd, 1H, *J* 12.0 Hz, H-6a^I), 4.94–5.01 (m, 2H, H-3^I, H-6b^I), 5.21 (d, 1H, *J* 2.0 Hz, H-1^{II}), 5.37 (dd, 1H, *J* 9.0, 10.2 Hz, H-2^I), 5.52 (dd, 1H, *J* 2.0, 3.2 Hz, H-2^{II}), 5.66 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.75 (dd, 1H, *J* 3.2, 10.0 Hz, H-3^{II}), 7.26–8.10 (m, 20H, 4*Ph*). Anal. Calcd for $C_{46}H_{46}O_{15}S$: C, 63.44; H, 5.32. Found: C, 63.71; H, 5.20.

3.4. 4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (15)

To a solution of **13** (5.04 g, 17.6 mmol) in pyridine (50 mL) was added TBSCl (3.2 g, 21.2 mmol) at 0 °C. The mixture was stirred at rt for 2.5 h, then Ac_2O (12 mL) was added. The mixture was stirred at rt for another 4 h, then co-evaporated with toluene to dryness under reduced pressure. The residue was purified by column chromatography (3:1 petroleum ether–EtOAc) to give **14** as a solid (8.33 g, 90%). To a solution of the above solid (8.02 g, 15.2 mmol) in dry CH_2Cl_2 (80 mL) was added $BF_3{\cdot}Et_2O$ (5.4 mL, 41.8 mmol). The mixture was stirred at rt for 40 min, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated the

reaction complete. The mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ and then satd aq NaCl. The organic layer was combined, dried and concentrated. Purification by column chromatography (1:1 petroleum ether–EtOAc) gave **15** as a white solid (5.91 g, 94%): $[\alpha]_D^{25}$ −5 (*c* 4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03, 2.06, 2.07 (3s, 3 × 3H, 3C*H*$_3$CO), 2.17 (br s, 1H, O*H*), 3.61–3.66 (m, 2H, H-5, H-6a), 3.73–3.77 (m, 4H, H-6b, OC*H*$_3$), 5.01 (d, 1H, *J* 7.9 Hz, H-1), 5.10 (t, 1H, *J* 9.4 Hz, H-4), 5.21 (dd, 1H, *J* 7.9, 9.4 Hz, H-2), 5.32 (t, 1H, *J* 9.4 Hz, H-3), 6.81–6.94 (m, 4H, *Ph*). Anal. Calcd for $C_{19}H_{24}O_{10}$: C, 55.34; H, 5.87. Found: C, 55.08; H, 5.95.

3.5. 4-Methoxyphenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (16)

To a mixture of compounds **12** (1.10 g, 1.26 mmol) and **15** (474 mg, 1.15 mmol) in anhyd CH_2Cl_2 (12 mL) was added NIS (425 mg, 1.89 mmol) and TMSOTf (23 μL, 0.13 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 30 min, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that all starting materials were consumed. The reaction mixture was neutralized with Et_3N, then concentrated. Column chromatography (3:2 petroleum ether–EtOAc) of the residue gave **16** as a foamy solid (1.206 g, 86%): $[\alpha]_D^{25}$ +75 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (d, 3H, *J* 6.2 Hz, H-6^{III}), 1.89, 2.01, 2.02, 2.06, 2.08 (5s, 5 × 3H, 5Ac), 3.70 (dd, 1H, *J* 5.3, 11.3 Hz, H-6a^{I}), 3.75–3.78 (m, 4H, H-5^{II}, OC*H*$_3$), 3.84–3.88 (m, 2H, H-5^{I}, H-6b^{I}), 4.06 (t, 1H, *J* 9.4 Hz, H-4^{II}), 4.15–4.19 (m, 1H, H-5^{III}), 4.61 (dd, 1H, *J* 4.0, 12.4 Hz, H-6a^{II}), 4.67 (d, 1H, *J* 7.9 Hz, H-1^{II}), 4.90–5.01 (m, 4H, H-2^{I}, H-6b^{II}, H-3^{II}, H-1^{I}), 5.19–5.32 (m, 4H, H-1^{III}, H-4^{I}, H-2^{II}, H-3^{I}), 5.52 (t, 1H, *J* 2.1 Hz, H-2^{III}), 5.68 (t, 1H, *J* 9.7 Hz, H-4^{III}), 5.74 (dd, 1H, *J* 2.1, 9.7 Hz, H-3^{III}), 6.88–8.09 (m, 24H, *Ph*). Anal. Calcd for $C_{63}H_{64}O_{25}$: C, 61.96; H, 5.28. Found: C, 62.25; H, 5.16.

3.6. 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (4)

To a solution of **16** (1.10 g, 0.90 mmol) in 4:1 CH_3CN–H_2O (v/v, 20 mL) was added CAN (1.41 g, 2.7 mmol), and the mixture was stirred at rt for 30 min, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was extracted with EtOAc, and the extract was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (3:2 petroleum ether–EtOAc) to afford a solid. To a mixture of the solid in CH_2Cl_2 (8 mL) were added trichloroacetonitrile (0.4 mL) and DBU (0.04 mL). The reaction mixture was stirred at rt for 1.5 h and then concentrated in vacuo. The residue was purified by column chromatography (2:1 petroleum ether–EtOAc) to give **4** as a white foamy solid (0.91 g, 80%): $[\alpha]_D^{25}$ +131 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (d, 3H, *J* 6.2 Hz, H-6^{III}), 2.00, 2.02, 2.03, 2.07, 2.08 (5s, 5 × 3H, 5Ac), 3.60 (dd, 1H, *J* 5.2, 11.3 Hz, H-6b^{I}), 3.87 (m, 1H, H-5^{II}), 3.96 (dd, 1H, *J* 2.0, 11.3 Hz, H-6a^{I}), 4.07 (t, 1H, *J* 9.2 Hz, H-4^{II}), 4.15–4.19 (m, 2H, H-5^{I}, H-5^{III}), 4.63 (dd, 1H, *J* 4.0, 12.4 Hz, H-6b^{II}), 4.66 (d, 1H, *J* 7.9 Hz, H-1^{II}), 4.90 (dd, 1H, *J* 7.9, 9.2 Hz, H-2^{II}), 4.93 (dd, 1H, *J* 3.6, 9.5 Hz, H-2^{I}), 5.05–5.08 (m, 2H, H-3^{II}, H-6a^{II}), 5.20 (d, 1H, *J* 1.8 Hz, H-1^{III}), 5.33 (t, 1H, *J* 9.5 Hz, H-3^{I}), 5.51–5.56 (m, 2H, H-4^{I}, H-2^{III}), 5.65 (t, 1H, *J* 10.0 Hz, H-4^{III}), 5.75 (dd, 1H, *J* 3.2, 10.0 Hz, H-3^{III}), 6.53 (d, 1H, *J* 3.6 Hz, H-1^{I}), 7.23–8.09 (m, 20H, 4*Ph*), 8.69 (s, 1H, N*H*). Anal. Calcd for $C_{58}H_{58}Cl_3NO_{24}$: C, 55.31; H, 4.64. Found: C, 55.53; H, 4.58.

3.7. 3,4-Di-*O*-benzoyl-1,2-*O*-methoxyethylidene-α-D-xylopyranose (22)

To a mixture of **21** (10.02 g, 34.5 mmol) in MeOH (80 mL) was added 1.0 M NaOMe–MeOH. The pH of the mixture was kept at 10 at rt for 2.5 h, TLC (4:1 EtOAc–MeOH) indicated that the reaction was complete. The reaction mixture was concentrated, and the residue was purified by column chromatography (EtOAc) to give a syrup. The syrup was dissolved in pyridine (40 mL), and BzCl (8.82 mL, 75.9 mmol) in pyridine (15 mL) was added dropwise to the mixture cooled in an ice-water bath. The mixture was stirred at rt for 4 h, and TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated with toluene and purified by column chromatography (4:1 petroleum ether–EtOAc) to give **22** as a syrup (12.8 g, 90%): $[\alpha]_D^{25}$ +30 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 3H, C*H*$_3$), 3.32 (s, 3H, OC*H*$_3$), 3.97 (dd, 1H, *J* 6.0, 12.5 Hz, H-5a), 4.16 (dd, 1H, *J* 4.7, 12.5 Hz, H-5b), 4.41–4.43 (m, 1H, H-2), 5.21–5.22 (m, 1H, H-4), 5.69–5.72 (m, 2H, H-3, H-1), 7.41–8.09 (m, 10H, 2*Ph*). Anal. Calcd for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.51; H, 5.42.

3.8. Isopropyl 3,4-di-*O*-benzoyl-1-thio-β-D-xylopyranoside (24)

To a solution of Me_2CHSH (1.2 mL, 11.02 mmol) and TMSOTf (181 μL, 1 mmol) in anhyd CH_2Cl_2 (25 mL) was slowly added a solution of compound **22** (3.726 g, 9 mmol) in anhyd CH_2Cl_2 (10 mL) under an N_2 atmosphere at 0 °C. The mixture was stirred under these

conditions for 25 min, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that all starting materials were consumed. The reaction mixture was concentrated and dissolved in 1:1 CH_2Cl_2–MeOH (100 mL) co-solvent containing 5% HCl. The mixture was stirred at rt for 16 h, evaporated under reduced pressure at rt for 5 min, and then concentrated to dryness at 45 °C. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave **24** as a syrup (2.27 g, 55% for two steps): $[\alpha]_D^{25}$ +111 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04 (br s, 1H, *OH*), 1.36–1.39 (2d, 6H, *J* 1.2 Hz, $CH(CH_3)_2$), 3.21–3.28 (m, 1H, $CH(CH_3)_2$), 3.55 (dd, 1H, *J* 9.4, 11.5 Hz, H-5a), 3.77 (t, 1H, *J* 8.4 Hz, H-2), 4.42 (dd, 1H, *J* 5.1, 11.4 Hz, H-5e), 4.65 (d, 1H, H-1, *J* 8.8 Hz), 5.29–5.35 (m, 1H, H-4), 5.58 (t, 1H, *J* 8.6 Hz, H-3), 7.37–8.05 (m, 10H, 2*Ph*). Anal. Calcd for $C_{22}H_{24}O_6S$: C, 63.44; H, 5.81. Found: C, 63.09; H, 5.75.

3.9. 3-*O*-[3,4-Di-*O*-benzoyl-β-D-xylopyranosyl]oleanolic acid 28-*O*-trityl ester (25)

To a mixture of compounds **24** (800 mg, 1.92 mmol) and **3** (1.10 g, 1.57 mmol) in anhyd CH_2Cl_2 (30 mL) was added NIS (440 mg, 1.96 mmol) and TMSOTf (36 μL, 0.20 mmol) under an N_2 atmosphere at −42 °C. The mixture was stirred under these conditions for 1.5 h, quenched by Et_3N, diluted with CH_2Cl_2 (20 mL), and washed with aq $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (7:1 petroleum ether–EtOAc) to give compound **25** as a white foamy solid (1.14 g, 70%): $[\alpha]_D^{25}$ +40 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.33, 0.72, 0.80, 0.87, 0.89, 0.99, 1.10 (7s, 7 × 3H, 7C*H*$_3$), 2.48 (br d, 1H, *J* 3.1 Hz, O*H*), 2.84 (dd, 1H, *J* 4.1, 13.0 Hz, H-18), 3.20 (dd, 1H, *J* 4.6, 11.5 Hz, H-3 of oleanolic acid), 3.49 (dd, 1H, *J* 9.3, 11.6 Hz, H-5a), 3.78–3.83 (m, 1H, H-2), 4.31 (dd, 1H, *J* 5.2, 11.6 Hz, H-5b), 4.55 (d, 1H, *J* 6.9 Hz, H-1), 5.10 (t, 1H, *J* 3.2 Hz, H-12 of oleanolic acid), 5.27–5.32 (m, 1H, H-4), 5.57 (t, 1H, *J* 8.9 Hz, H-3), 7.20–8.01 (m, 25H, 5*Ph*). Anal. Calcd for $C_{68}H_{78}O_9$: C, 78.58; H, 7.56. Found: C, 78.23; H, 7.41.

3.10. 3-*O*-[2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-β-D-xylopyranosyl]oleanolic acid 28-*O*-trityl ester (26)

To a mixture of compounds **6** (574 mg, 0.92 mmol) and **25** (800 mg, 0.77 mmol) in anhyd CH_2Cl_2 (12 mL) was added TMSOTf (18 μL, 0.1 mmol) under an N_2 atmosphere at −42 °C. The mixture was stirred under these conditions for 40 min, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (6:1 petroleum ether–EtOAc) to give compound **26** as a white foamy solid (865 mg, 75%): $[\alpha]_D^{25}$ +113 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.35, 0.82, 0.88, 0.90, 0.91, 1.11, 1.29, 1.31 (8s, 8 × 3H, 8C*H*$_3$), 2.83 (dd, 1H, *J* 4.1, 13.0 Hz, H-18 of oleanolic acid), 3.25 (dd, 1H, *J* 4.4, 11.6 Hz, H-3 of oleanolic acid), 3.66 (dd, 1H, *J* 7.1, 11.9 Hz, H-5a^I), 4.12 (t, 1H, *J* 7.5 Hz, H-2^I), 4.40 (dd, 1H, *J* 4.2, 11.9 Hz, H-5b^I), 4.49 (dd, 1H, *J* 6.2, 9.9 Hz, H-5^{II}), 4.87 (d, 1H, *J* 7.5 Hz, H-1^I), 5.23–5.34 (m, 2H, H-4^I, H-12 of oleanolic acid), 5.34 (d, 1H, *J* 1.6 Hz, H-1^{II}), 5.57 (dd, 1H, *J* 1.6, 3.5 Hz, H-2^{II}), 5.60 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.70 (t, 1H, *J* 7.5 Hz, H-3^I), 5.81 (dd, 1H, *J* 3.5, 10.0 Hz, H-3^{II}), 7.21–8.04 (m, 40H, 8*Ph*). Anal. Calcd for $C_{95}H_{100}O_{16}$: C, 76.18; H, 6.73. Found: C, 75.87; H, 6.61.

3.11. 3-*O*-[2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-β-D-xylopyranosyl]oleanolic acid (27)

Compound **26** (800 mg, 0.53 mmol) was dissolved in 80% aq acetic acid (10 mL). The mixture was stirred at 70 °C for 1 h and then co-evaporated with toluene to dryness under reduced pressure. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give compound **27** as a foamy solid (616 mg, 92%): $[\alpha]_D^{25}$ +72 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.35, 0.82, 0.88, 0.90, 0.91, 1.11, 1.29, 1.31 (8s, 8 × 3H, 8C*H*$_3$), 2.83 (dd, 1H, *J* 4.1, 13.0 Hz, H-18 of oleanolic acid), 3.25 (dd, 1H, *J* 4.4, 11.6 Hz, H-3 of oleanolic acid), 3.66 (dd, 1H, *J* 6.9, 12.0 Hz, H-5a^I), 4.12 (t, 1H, *J* 7.1 Hz, H-2^I), 4.40 (dd, 1H, *J* 4.2, 12.0 Hz, H-5b^I), 4.49 (m, 1H, H-5^{II}), 4.87 (d, 1H, *J* 7.1 Hz, H-1^I), 5.20–5.25 (m, 1H, H-4^I), 5.29 (t, 1H, *J* 3.2 Hz, H-12 of oleanolic acid), 5.34 (d, 1H, *J* 1.6 Hz, H-1^{II}), 5.57 (dd, 1H, *J* 1.6, 3.5 Hz, H-2^{II}), 5.60 (t, 1H, *J* 10.0 Hz, H-4^{II}), 5.70 (t, 1H, *J* 7.1 Hz, H-3^I), 5.82 (dd, 1H, *J* 3.5, 10.0 Hz, H-3^{II}), 7.21–8.04 (m, 25H, 5*Ph*). Anal. Calcd for $C_{76}H_{86}O_{16}$: C, 72.71; H, 6.90. Found: C, 72.46; H, 6.99.

3.12. 3-*O*-[2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-β-D-xylopyranosyl]oleanolic acid 28-*O*-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl] ester (28)

To a mixture of compounds **27** (600 mg, 0.48 mmol) and trisaccharide **4** (730 mg, 0.58 mmol) in anhyd CH_2Cl_2 (15 mL) was added TMSOTf (11 μL, 0.06 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 30 min, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (3:2 petroleum ether–EtOAc) to give compound **28** as a white foamy solid (880 mg, 78%): $[\alpha]_D^{25}$ +81 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76, 0.85, 0.89, 0.91, 0.95, 1.12,

1.13, 1.27, 1.29 (9s, 9 × 3H, 9CH_3), 2.00, 2.02, 2.03, 2.07, 2.08 (5s, 5 × 3H, 5Ac), 2.81 (dd, 1H, *J* 4.1, 13.0 Hz, H-18 of oleanolic acid), 3.10 (br s, 1H), 3.26 (dd, 1H, *J* 4.3, 11.3 Hz, H-3 of oleanolic acid), 3.67–3.58 (m, 2H), 3.90–3.79 (m, 3H), 4.18–4.04 (m, 3H), 4.41 (dd, 1H, *J* 4.2, 11.9 Hz, H-5^{IV}), 4.54–4.45 (m, 1H, H-5^{III}), 4.65–4.61 (m, 2H), 5.00–4.87 (m, 4H), 5.25–5.13 (m, 4H), 5.34–5.29 (m, 3H), 5.63–5.51 (m, 4H, H-2^{III}, H-2^{V}, H-4^{I}, H-4^{V}), 5.70–5.65 (m, 2H, H-3^{IV}, H-4^{III}), 5.73 (dd, 1H, *J* 3.2, 10.2 Hz, H-3^{V}), 5.82 (dd, 1H, *J* 3.5, 10.1 Hz, H-3^{III}), 7.23–8.20 (m, 45H, 9*Ph*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 170.16, 170.14, 169.9, 169.3, 168.8, 165.8, 165.79, 165.70, 165.48, 165.42, 165.36, 165.23, 165.06, 164.93, 142.0, 133.4, 133.36, 133.26, 133.226, 133.10, 133.00, 132.9, 132.8, 129.88, 129.85, 129.79, 129.73, 129.70, 129.61, 129.54, 129.27, 129.24, 129.22, 129.18, 129.08, 128.51, 128.42, 128.39, 128.36, 128.33, 128.24, 128.17, 128.14, 103.5, 100.1, 98.5, 97.5, 92.1, 89.5, 77.22, 74.14, 73.9, 73.8, 72.96, 72.91, 72.14, 71.87, 71.80, 71.36, 71.10, 70.52, 70.01, 69.60, 69.36, 68.79, 68.04, 67.56, 67.24, 62.31, 61.16, 55.67, 47.64, 46.70, 45.81, 41.74, 41.05, 39.31, 39.29, 39.21, 38.77, 36.77, 33.74, 32.94, 31.65, 30.54, 29.66, 28.02, 27.86, 26.01, 25.56, 23.47, 23.40, 22.87, 22.59, 21.09, 20.65, 20.63, 20.58, 20.55, 20.54, 18.14, 17.41, 17.00, 16.54, 15.50. Anal. Calcd for $C_{132}H_{142}O_{39}$: C, 67.39; H, 6.08. Found: C, 67.71; H, 6.15. MALDI-TOF-MS: calcd for $C_{132}H_{142}O_{39}$: 2351 $[M]^+$; found, 2373.8 $[M+Na]^+$.

3.13. 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]oleanolic acid 28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (1)

Compound **28** (400 mg, 0.17 mmol) was dissolved in anhyd 2:1 CH_2Cl_2–MeOH (30 mL), and then 1.0 M NaOMe in MeOH (0.4 mL) was added at 0 °C. The mixture was stirred at rt for 7 h, at the end of which time TLC (2:1:0.5 *n*-BuOH–EtOH–H_2O) indicated that all starting materials were consumed. The solution was neutralized with ion-exchange resin (H^+), and then filtered and concentrated. The residue was purified on a Bio-Gel P2 column using H_2O as eluent, and the desired fractions were combined and freeze dried to afford **1** as an amorphous solid (197 mg, 96%): $[\alpha]_D^{25}$ +8.6 (*c* 1, MeOH); ^{1}H NMR (400 MHz, C_6D_5N): δ 0.87 (m, 9H, 3CH_3), 1.08 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.69 (d, 3H, *J* 4.8 Hz, H-6^{III}), 1.70 (d, 3H, *J* 5.4 Hz, H-6^{V}), 3.20 (m, 1H, H-3 of oleanolic acid), 4.82 (d, 1H, *J* 7.3 Hz, H-1^{IV}), 4.98 (d, 1H, *J* 7.8 Hz, H-1^{I}), 5.85 (br s, 1H, H-1^{III}), 6.23 (d, 1H, *J* 7.8 Hz, H-1^{II}), 6.53 (br s, 1H, H-1^{V}). ^{13}C NMR (100 MHz, C_6D_5N): δ 176.4, 144.0, 122.8, 106.0, 104.8, 102.6, 101.8, 95.6, 88.4, 79.5, 78.6, 78.1, 78.0, 77.8, 77.1, 76.4, 75.3, 75.3, 74.0, 73.9, 72.7, 72.5, 72.3, 71.4, 70.7, 69.7, 69.1, 66.9, 61.2, 38.9, 26.8, 39.5, 56.0, 18.5, 18.6, 33.0, 39.8, 48.0, 36.9, 23.7, 42.0, 28.2, 23.3, 46.9, 41.6, 46.1, 30.7, 33.9, 32.4, 27.9, 17.0, 15.6, 17.4, 26.0, 23.6. MALDITOF-MS: calcd for $C_{59}H_{96}O_{25}$: 1204 $[M]^+$; found, 1226.6 $[M+Na]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.11.015.

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