Carbohydrate Research 346 (2011) 1786–1791

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis of stryphnoside A, a triterpene saponin isolated from the pericarps of *Stryphnodendron fissuratum*

Xun Lv [a,b], Shouyi Yu [a,b], Jing Wang [a], Yuguo Du [a,b,*]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] College of Chemistry and Chemical Engineering, Graduate University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 9 April 2011
Received in revised form 3 June 2011
Accepted 6 June 2011
Available online 12 June 2011

Keywords:
Stryphnosides
Glycosylation
Saponins
Natural products
Cytotoxicity

ABSTRACT

Stryphnoside A, α-L-rhamnopyranosyl 3β-*O*-[α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oate, has been synthesized in 11 steps in 15% overall yield starting from the naturally abundant oleanolic acid. Condensation of a partially protected glucopyranosyl donor and 2α,3β-dihydroxyolean-12-en-28-oic acid derivative using inverse glycosylation procedure has significantly simplified the target saponin synthesis. Stryphnoside A exhibited weak cytotoxic activities against tumor cells HeLa, A549, and HepG2 with IC_{50} at mM level.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

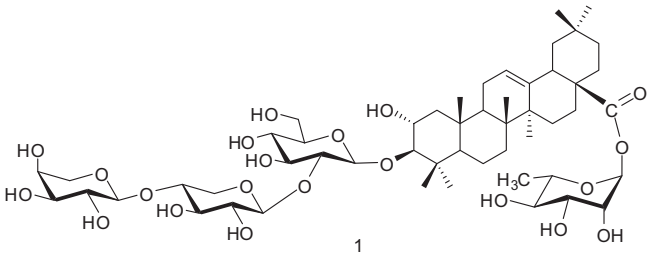
Saponins, as an important family of natural products, are widely distributed in terrestrial plants and some lower marine animals. The diversity of structures, the challenges of isolation and purification, the broad spectrum of biological and pharmacological activities have driven chemists to the research field of saponins.[1–5] Stryphnoside A (**1**, Fig. 1) was originally isolated from the pericarps of *stryphnodendron fissuratum*, part of the *stryphnodendron* family where species have long been used as medicinal plants for the treatment of hemorrhages, wounds, and diarrhea in Brazil.[6] Interestingly, it was also suggested that the fruit of this Brazilian plant could cause bovine death.[7] On the basis of spectroscopic analysis and hydrolytic reactions, the chemical structure of stryphnoside A was elucidated as α-L-rhamnopyranosyl 3β-*O*-[α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oate (**1**).[8] The structural complexity, especially having a C-2α hydroxyl group and C-3β/C-28 diglycosides on oleanolic acid derivative, has hindered the chemical synthesis, and the successful examples are rarely reported.[9,10] Curious about the toxic activities of stryphnoside A, as well as an intention to explore a practical synthetic strategy for the structure-related compounds, we launched a project toward the total synthesis of stryphnoside A.

In our previous research, we found that a thioglycoside having a C-2 unprotected hydroxyl group could be a good glycosyl donor to obtain β glycosylation product, and at the same time facilitate the continuous C-2 glycosylation.[11,12] Herein, we report the total synthesis of stryphnoside A applying this methodology.

2. Results and discussion

There are two challenges in stryphnoside A synthesis, namely, β-D-glucopyranosylation on C-3 of 2α,3β-dihydroxyolean-12-en-28-oic acid derivative, and the following C-2′ sugar chain elongation on glucose residue. To provide a suitable aglycon acceptor, 2α-hydroxy-3-oxoolean-12-en-28-oic acid benzyl ester (**2**) was prepared from the natural oleanolic acid according to a published method (Scheme 1).[13]

Acetylation of **2** with Ac_2O in pyridine (→**3a**), followed by $NaBH_4$ reduction, afforded benzyl-2α-acetyl-3β-hydroxyolean-12-en-28-oic acid (**4a**) in 78% yield. Glycosylation of **4a** and

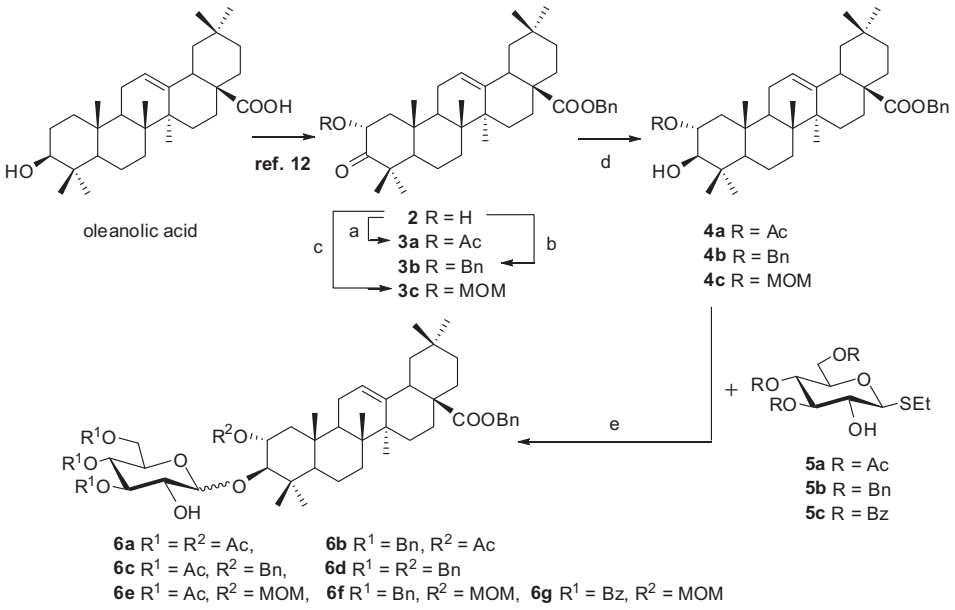


1

Figure 1. Chemical structure of stryphnoside A.

* Corresponding author. Tel./fax: +86 10 62849126.
E-mail addresses: duyuguo@rcees.ac.cn, duyuguo@mail.rcees.ac.cn (Y. Du).

0008-6215/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2011.06.006



Scheme 1. Regents and conditions: (a) Ac_2O, Pyr, rt, 6 h, 96%; (b) $BnOC(NH)CCl_3$, TfOH, CH_2Cl_2, 0 °C, 91%; (c) $^iPr_2EtN$, CH_3OCH_2Cl, CH_2Cl_2, 0 °C→rt, 4 h, 88%; (d) $NaBH_4$, THF/EtOH; −20 °C, 48 h, 78% for **4a**; rt, 20 h, 84% for **4b**; rt, 16 h, 82% for **4c**; (e) NIS, TMSOTf, CH_2Cl_2, −42 °C, 30 min, 36–87%.

ethyl 3,4,6-tri-*O*-acetyl-β-D-thioglucopyranoside (**5a**)[14] in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) at −42 °C in CH_2Cl_2 gave messy products. However, the same reaction proceeded smoothly with 'inverse procedure',[15] that is, donor **5a** and NIS in dry CH_2Cl_2 was added into a pre-cooled mixture of acceptor **4a** and TMSOTf in CH_2Cl_2, generating a major component in 66% yield, which was carefully identified as the α-anomer (δ 5.07 ppm, J 3.9 Hz, H-1). Surprised by this observation, we then studied the stereo outcomes of this reaction using different protecting groups on both glycosyl donor and acceptor. The results were summarized in Table 1. We found that coupling reaction between 2α-methoxylmethylated (MOM) oleanolic acid derivative **4c** and benzylated thioglycoside **5b**[16] gave the best β-selectivity and good yield under our conditions (entry 6, **6f**).

With compound **6f** in hand, we started a formal convergent total synthesis of stryphnoside A (Scheme 2). Zinc chloride promoted intramolecular rearrangement of the thio ortho ester **7**[17] gave ethyl 2-*O*-acetyl-β-D-thioxylopyranoside (**8**) in an excellent yield of 92%. TMSOTf catalyzed regioselective[18] glycosylation of diol **8** and 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl trichloroacetimidate (**9**)[19] was applied in dry methylene dichloride at −42 °C with inverse procedure (→**10**), followed by acetylation with acetic anhydride in pyridine, furnished disaccharide donor ethyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-thioxylopyranoside (**11**) in 73% isolated yield over two steps. Based on 2D NMR spectrum, H-1 of arabinose residue appeared at 4.50 ppm (J 5.5 Hz), while a multiple peak at δ 3.85 ppm corresponding to H-4 of xylose, confirming the right structure of **11**. Coupling of **11** and **6f** in dry CH_2Cl_2 at low temperature (−50 °C) was carried out smoothly in the presence of co-catalyst NIS/TMSOTf, providing the desired saponin derivative **12** in a good yield of 79%. Selective removal of MOM group[20] from **12** with catalytic amount of *p*-toluenesulfonic acid (→**13**, 86%), followed by H_2 hydrogenation over $Pd(OH)_2/C$ (→**14**, 91%) and acetylation with Ac_2O-pyridine in CH_2Cl_2, afforded 3β-*O*-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-2α-hydroxyloleanolic acid (**15**) in a yield of 70% over three steps. It was interesting that 2α-OH of oleanolic acid derivative **15** could not be acetylated under the current acetylation conditions. Condensation of acid **15** and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**16**)[21] in dry CH_2Cl_2 at −78 °C using TMSOTf-catalyzed inverse procedure obtained acetylated stryphnoside A derivatives **17** (79%). Global deacetylation of **17** with catalytic amount of sodium methoxide in CH_2Cl_2–MeOH co-solvent finished the total synthesis of stryphnoside A (**1**). Remarkably, this complex natural saponin was prepared convergently in 11 steps and in 15% overall yield. The characteristic data (^1H, ^{13}C NMR, MS, and optical rotation) of the synthetic compound **1** were identical to those reported for the natural product.[8]

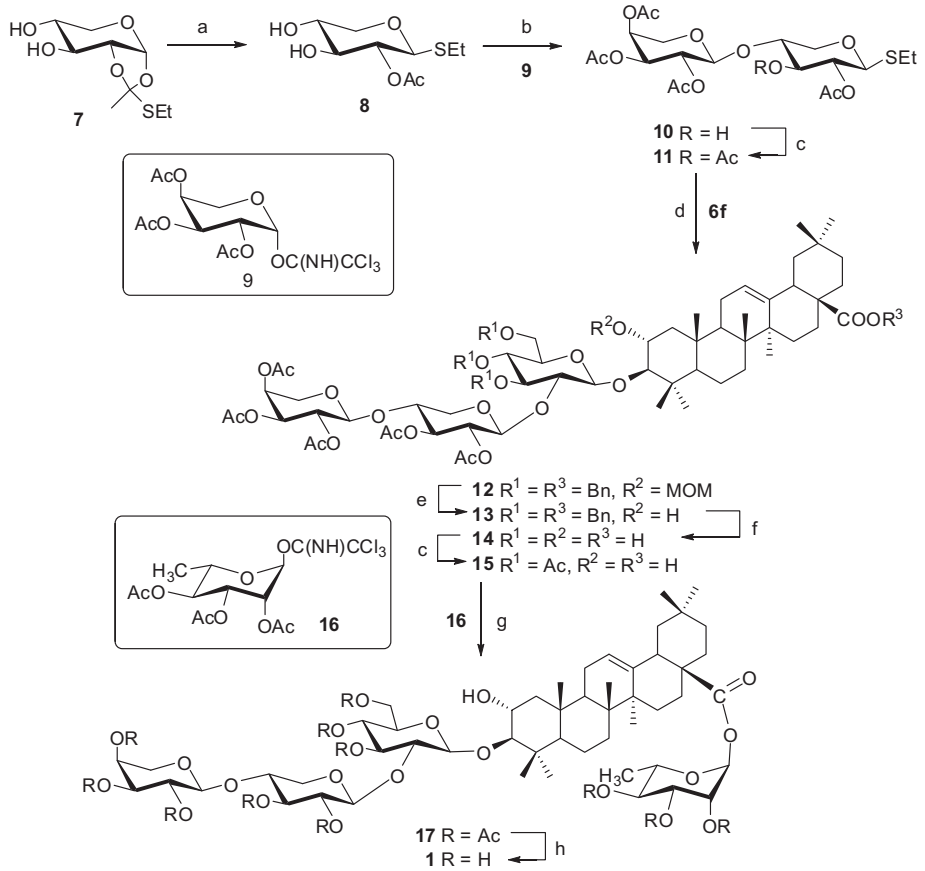
The cytotoxic activities of synthetic compound **1** on tumor cells HeLa, A549, and HepG2, as well as two normal cell lines (HL7702 and H9C2), were evaluated following the standard MTT assay.[22] As shown in Table 2, stryphnoside A (**1**) inhibited tumor cell growth with IC_{50} ranging from 3.2 to 4.8 mM, while the corresponding positive control showed inhibition at μM scale. Interestingly, compound **1** exhibited a lower cytotoxicity to the normal cells HL7702 and H9C2.

In conclusion, the natural product stryphnoside A has been chemically synthesized in 11 steps in 15% overall yield starting from the natural abundant oleanolic acid and monosaccharides. Applying a partially protected glucopyranosyl donor, combining with inverse glycosylation procedure, has significantly simplified the target saponin synthesis. The approach described here should be valuable for structure-related[8,13,23–26] molecule design, synthesis, and bioactivity screening.

Table 1
Synthesis of the key intermediate **6**

Entry	Donor	Acceptor	Product	Yield[a] (%)	α:β
1	**5a**	**4a**	**6a**	73	66:7
2	**5b**	**4a**	**6b**	70	61:9
3	**5a**	**4b**	**6c**	52	26:26
4	**5b**	**4b**	**6d**	60	30:30
5	**5a**	**4c**	**6e**	36	5:31
6	**5b**	**4c**	**6f**	87	9:78
7	**5c**	**4c**	**6g**	62	10:52

[a] Isolated total yield.



Scheme 2. Complete the total synthesis of stryphnoside A (**1**). Reagents and conditions: (a) $ZnCl_2$, CH_2Cl_2, −78 °C, 92%; (b) TMSOTf, CH_2Cl_2, −15 °C, then **9**; (c) Ac_2O, Py; for **11**, 73% from **8**; for **15**, 81% from **13**; (d) NIS, TMSOTf, CH_2Cl_2, −50 °C, 79%; (e) TsOH, CH_3OH, 86%; (f) $Pd(OH)_2/C$, H_2; (g) TMSOTf, CH_2Cl_2, −78 °C, then **16**, 79%; (g) CH_3ONa, CH_3OH–CH_2Cl_2, 91%.

Table 2
Cytotoxicity of compound **1** on tumor cells and normal cells (IC_{50})[a]

Entry	Hela	A549	HepG	HL7702	H9C2
Compound **1** (mM)	3.6	4.8	3.2	>6.0	>6.0
Cisplatin (μM)	26.1	>30.0	10.0	n.d.	n.d.

[a] Values are means of three independent experiments.

3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. ^1H NMR and ^{13}C NMR were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or CD_3OD. 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. Benzyl-2α-*O*-methoxymethyl-3-oxoolean-12-en-28-oic acid (3c)

To a stirred mixture of **2** (561 mg, 1.0 mmol) and *N*,*N*-diisopropylethylamine (0.52 mL, 3.0 mmol) in CH_2Cl_2 (30 mL) was added dropwise chloromethyl methyl ether (0.44 mL, 6.0 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and at rt for 3 h, then quenched by addition of water and extracted with ether (30 mL × 3). The combined organic layer was washed successively with cold 0.2 N HCl, satd $NaHCO_3$ solution, and water, then dried over Na_2SO_4, and concentrated under diminished pressure. The residue was purified by silica gel chromatography (30:1 petroleum ether–EtOAc) to give **3c** (532 mg, 88%): $[\alpha]_D^{25}$ +311 (*c* 2.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.35–7.30 (m, 5H, Ph*H*), 5.28 (t, 1H, *J* 3.5 Hz, H-12), 5.10, 5.06 (2d, 2H, 12.5 Hz, PhCH_2), 4.70 (s, 2H, CH_3OCH_2), 4.54 (dd, 1H, *J* 6.0, 12.8 Hz, H-2), 3.38 (s, 3H, CH_3OCH_2), 2.22 (dd, 1H, *J* 3.8, 4.3 Hz, H-18), 2.01–0.55 (m, 41H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 213.1, 177.3, 143.9, 136.4, 128.4, 128.0, 127.9, 121.9, 95.4, 65.9, 56.9, 55.6, 48.7, 47.4, 47.3, 46.6, 45.8, 41.7, 41.3, 39.3, 37.8, 33.8, 33.3, 32.3, 30.7, 27.5, 26.0, 25.8, 23.6, 22.9, 21.6, 19.2, 16.7, 16.1. Anal. Calcd for $C_{39}H_{56}O_5$: C, 77.44; H, 9.33. Found: C, 77.62; H, 9.42.

3.3. Benzyl-2α-*O*-methoxymethyl-3β-hydroxyolean-12-en-28-oic acid (4c)

To a solution of **3c** (605 mg, 1.0 mol) in THF (20 mL) and ethanol (4 mL) was added $NaBH_4$ (45 mg, 1.2 mmol) at 0 °C. After the mixture was stirred at 0 °C for 12 h, 1 N HCl (10 mL) was added dropwise, and the mixture was extracted with EtOAc (50 mL × 3). The organic layer was washed with saturated $NaHCO_3$ (30 mL × 3) and brine (30 mL × 3), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel chromatography (30:1 petroleum ether–EtOAc) to give **4c** as a

syrup (498 mg, 82%): [α_D^{25} +129 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.30 (m, 5H, Ph*H*), 5.28 (t, 1H, *J* 3.5 Hz, H-12), 5.10, 5.04 (2d, 2H, 12.5 Hz, PhCH_2), 4.73, 4.69 (2d, 2H, *J* 6.8 Hz, CH_3OCH_2), 3.54–3.52 (m, 1H, H-2), 3.40 (s, 3H, CH_3OCH_2), 3.07 (d, 1H, *J* 9.4 Hz, H-3), 2.92 (dd, 1H, *J* 3.8, 4.3 Hz, H-18), 1.12, 1.06, 0.93, 0.92, 0.90, 0.83, 0.59 (7s, 7 × 3H, 7CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 143.8, 136.4, 128.4, 128.0, 127.9, 122.2, 96.6, 81.3, 78.6, 65.9, 55.6, 54.9, 47.5, 46.7, 45.8, 44.5, 41.7, 41.3, 39.3, 39.1, 38.1, 33.8, 33.1, 32.6, 32.3, 30.7, 28.8, 27.5, 25.9, 23.6, 23.5, 23.0, 18.1, 17.0, 16.8, 16.5. Anal. Calcd for $C_{39}H_{58}O_5$: C, 77.19; H, 9.63. Found: C, 77.04; H, 9.72.

3.4. Benzyl-3β-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2α-*O*-methoxymethylolean-12-en-28-oic acid (6f)

To a solution of **4c** (61 mg, 0.10 mmol) containing 4 Å molecular sieves in anhyd CH_2Cl_2 (5 mL) was added TMSOTf (0.9 μL, 0.050 mmol) under N_2 atmosphere at −42 °C. After stirring for 10 min, a solution of **5b** (55 mg, 0.11 mmol) and NIS (37 mg, 0.16 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The mixture was stirred under these conditions for 20 min, quenched by Et_3N, diluted with CH_2Cl_2, 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 (3:1 petroleum ether–EtOAc) to give compound **6f** as a white foam (81 mg, 78%): [α_D^{25} +97 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.20 (m, 20H, 5Ph*H*), 5.29 (t, 1H, *J* 3.5 Hz, H-12), 5.10, 5.07 (2d, 2H, *J* 12.5 Hz, PhCH_2), 4.95–4.81 (m, 4H, 2PhCH_2), 4.61–4.55 (m, 4H), 4.48 (d, 1H, 7.2 Hz, H-1′), 3.77–3.68 (m, 3H), 3.61–3.56 (m, 3H), 3.46–3.44 (m, 1H, H-2′), 3.30 (s, 3H, CH_3OCH_2), 3.24 (d, 1H, *J* 9.5 Hz, H-3), 2.92 (dd, 1H, *J* 3.8, 4.3 Hz, H-18), 2.54 (d, 1H, *J* 1.6 Hz), 1.11, 1.08, 0.96, 0.93, 0.91, 0.87, 0.59 (7s, 7 × 3H, 7CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 143.7, 138.7, 138.4, 138.2, 136.4, 128.4, 128.3, 128.0, 127.9, 127.5, 122.3, 104.0, 97.6, 90.3, 84.9, 75.6, 75.2, 75.1, 74.9, 74.1, 73.4, 69.1, 65.9, 55.2, 54.9, 47.6, 46.3, 45.8, 41.7, 41.3, 40.7, 39.2, 39.1, 37.7, 33.8, 33.1, 32.6, 32.3, 30.7, 28.7, 27.5, 25.8, 23.6, 23.5, 23.0, 18.1, 18.0, 16.8, 16.5. Anal. Calcd for $C_{66}H_{86}O_{10}$: C, 76.27; H, 8.34. Found: C, 76.14; H, 8.50.

3.5. Ethyl 2-*O*-acetyl-1-thio-β-D-xylopyranoside (8)

To a solution of carefully dried **7** (2.4 g, 10.0 mmol) in dry CH_2Cl_2 (100 mL) was added zinc chloride (1 M in ether, 0.5 mL, 0.50 mmol) at −78 °C. The mixture was stirred at these conditions for 30 min, warmed up to 0 °C in 30 min, then quenched with saturated aqueous $NaHCO_3$ (50 mL). The organic layer was separated, the water phase was extracted with CH_2Cl_2 (20 mL × 3), and the combined organic phase was dried over Na_2SO_4 and concentrated to dryness. The syrup was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give **8** as a white amorphous solid (2.2 g, 92%): [α_D^{25} +135 (*c* 2.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.76 (t, 1H, *J* 9.1 Hz, H-2), 4.40 (d, 1H, *J* 9.4 Hz, H-1), 4.09–4.07 (m, 1H, H-4), 3.74–3.53 (m, 4H), 3.27 (t, 1H, *J* 10.1 Hz, H-3), 2.68–2.66 (m, 2H, SCH_2CH_3), 2.13 (s, 3H, Ac), 1.25 (t, 3H, SCH_2CH_3). Anal. Calcd for $C_9H_{16}O_5S$: C, 45.75; H, 6.83. Found: C, 45.61; H, 6.94.

3.6. Ethyl 2,3,4–tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-1-thio-β-D-xylopyranoside (11)

To a pre-cooled mixture of **8** (236 mg, 1.0 mmol) and 4 Å molecular sieves in anhyd CH_2Cl_2 (20 mL) was added TMSOTf (18 μL, 0.10 mmol) under N_2 atmosphere at −15 °C. After stirring at these conditions for 10 min, a solution of **9** (463 mg, 1.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred for another 30 min, quenched by Et_3N. The organic layer was separated and dried over Na_2SO_4 and concentrated. The syrup was treated with Ac_2O (3 mL) in pyridine (3 mL) for 2 h, then concentrated to dryness. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give disaccharide **11** as a white foam (392 mg, 73%): [α_D^{25} −159 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23–5.21 (m, 1H, H-4′), 5.20 (t, 1H, *J* 8.8 Hz, H-3′), 5.04–5.02 (m, 2H, H-3, H-2′), 4.92 (t, 1H, *J* 9.2 Hz, H-2), 4.50 (d, 1H, *J* 5.5 Hz, H-1′), 4.47 (d, 1H, *J* 9.2 Hz, H-1), 4.01 (dd, 1H, *J* 12.4, 5.0 Hz, H-5a′), 3.98 (dd, 1H, *J* 6.5, 11.6 Hz, H-5a), 3.86–3.84 (m, 1H, H-4), 3.61 (dd, 1H, *J* 12.4, 2.4 Hz, H-5b′), 3.34 (dd, 1H, *J* 3.2, 11.6 Hz, H-5b), 2.67–2.64 (m, 2H, SCH_2CH_3), 2.10–2.03 (5s, 15H, 5Ac), 1.26 (t, 3H, SCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.4, 170.3, 169.7, 100.0, 84.4, 78.0, 74.4, 74.1, 70.7, 70.4, 69.8, 69.0, 68.0, 67.2, 62.1, 24.9, 21.4, 21.2, 15.5. Anal. Calcd for $C_{22}H_{32}O_{13}S$: C, 49.25; H, 6.01. Found: C, 49.36; H, 5.90.

3.7. Benzyl-3β-*O*-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl]-2α-*O*-methoxymethylolean-12-en-28-oic acid (12)

To a mixture of **6f** (208 mg, 0.20 mmol), **11** (129 mg, 0.24 mmol), NIS (81 mg, 0.36 mmol), and 4 Å molecular sieves in anhyd CH_2Cl_2 (10 mL) was added TMSOTf (2.2 μL, 0.012 mmol) under N_2 protection at −50 °C. The mixture was stirred at these conditions for 40 min, quenched by Et_3N, diluted with CH_2Cl_2 (30 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 (2:1 petroleum ether–EtOAc) to give compound **12** as a white foamy solid (239 mg, 79%): [α_D^{25} +92 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.20 (m, 20H, 5Ph*H*), 5.30 (t, 1H, *J* 3.5 Hz, H-12), 5.22 (d, 2H, 1.5 Hz), 5.13–5.00 (m, 6H), 4.91–4.79 (m, 4H), 4.74 (d, 1H, *J* 10.0 Hz), 4.63–4.54 (m, 4H), 4.47 (d, 1H, *J* 3.2 Hz), 4.41 (d, 1H, *J* 7.6 Hz), 3.99–3.96 (m, 2H), 3.80–3.58 (m, 8H), 3.38 (d, 1H, *J* 7.7 Hz), 3.29 (s, 3H), 3.11 (t, 2H), 2.92 (dd, 1H, *J* 3.8, 4.3 Hz), 2.67–2.65 (m, 2H, SCH_2CH_3), 2.12–2.04 (5s, 5 × 3H, 5Ac), 1.12, 1.07, 0.96, 0.93, 0.91, 0.88, 0.60 (7s, 7 × 3H, 7CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 170.8, 170.7, 170.5, 170.0, 169.7, 144.3, 138.9, 138.6, 138.4, 137.0, 129.4, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 122.9, 103.9, 100.5, 100.4, 98.6, 93.0, 86.8, 79.1, 77.9, 76.2, 76.0, 75.4, 75.2, 74.5, 74.0, 73.8, 72.6, 70.0, 69.8, 69.5, 67.4, 66.5, 63.5, 62.4, 55.7, 48.2, 47.3, 46.4, 42.3, 41.9, 41.6, 39.9, 38.3, 34.4, 33.7, 33.2, 33.0, 31.3, 30.3, 30.2, 28.7, 28.1, 26.4, 24.2, 24.1, 23.6, 21.5, 21.3, 21.2, 18.7, 17.9, 17.4, 17.2. Anal. Calcd for $C_{86}H_{112}O_{23}$: C, 68.23; H, 7.46. Found: C, 67.98; H, 7.60.

3.8. Benzyl-3β-*O*-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oic acid (13)

A mixture of **12** (151 mg, 0.10 mmol) and TsOH (76 mg, 0.40 mmol) in MeOH (15 mL) was stirred at rt for 4 h, then quenched with Et_3N and concentrated. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give **13** as a foamy solid (126 mg, 86%): [α_D^{25} +20 (*c* 2.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.22 (m, 20H, 5Ph*H*), 5.26 (t, 1H, *J* 3.5 Hz, H-12), 5.18 (m, 1H), 5.08–4.94 (m, 6H), 4.87–4.82 (m, 2H), 4.74 (t, 2H, *J* 11.2 Hz), 4.57–4.52 (m, 2H), 4.45–4.42 (m, 2H), 4.28 (d, 1H, *J* 7.8 Hz), 3.95–3.87 (m, 2H), 3.80–3.70 (m, 3H), 3.64–3.54 (m, 5H), 3.48–3.46 (m, 1H), 3.11 (t, 1H, *J* 11.4 Hz), 2.88 (d, 1H, *J* 9.2 Hz), 2.07–1.98 (5s, 15H, 5 Ac), 1.07, 1.05, 0.91, 0.88, 0.86, 0.85, 0.57 (7s, 7 × 3H, 7CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 170.8, 170.7, 170.5, 169.9, 169.7, 144.2, 138.5, 138.3,

138.2, 137.0, 129.4, 129.1, 129.0, 128.9, 128.5, 128.4, 128.2, 122.9, 104.0, 101.0, 100.4, 97.5, 86.2, 78.6, 76.4, 76.1, 75.6, 75.3, 74.3, 73.9, 72.6, 69.9, 69.8, 69.1, 67.3, 67.0, 66.5, 63.8, 62.3, 56.1, 48.2, 47.3, 46.9, 46.4, 42.2, 41.9, 41.1, 39.9, 38.1, 34.5, 33.7, 33.2, 33.0, 31.3, 30.3, 29.9, 28.6, 28.2, 26.8, 24.2, 24.0, 23.6, 21.5, 21.4, 21.3, 21.2, 20.1, 18.8, 17.8, 17.5, 17.7. MALDITOF–MS: calcd for $C_{56}H_{84}O_{22}$: 1468.7 $[M]^+$; found, 1491.9 $[M+Na]^+$, 1507.9 $[M+K]^+$. Anal. Calcd for $C_{84}H_{108}O_{22}$: C, 68.64; H, 7.41. Found: C, 68.82; H, 7.54.

3.9. 3β-*O*-[2,3,4-Tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oic acid (15)

To a solution of **13** (180 mg, 0.12 mmol) in EtOAc/ethanol (60 mL, v/v 1:1) at rt was added $Pd(OH)_2/C$ (60 mg). The suspension was stirred under hydrogen pressure (3 atm) for 0.5 h and then filtered. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (EtOAc) to afford **14** as a foamy solid. MALDITOF-MS: calcd for $C_{56}H_{84}O_{22}$: 1108.5 $[M]^+$; found, 1131.8 $[M+Na]^+$, 1147.8 $[M+K]^+$. To the above intermediate **14** (112 mg, 0.10 mmol) in CH_2Cl_2 (20 mL) was added pyridine (5 mL) and Ac_2O (3 mL) at rt. The solution was stirred at rt for 18 h and concentrated to dryness with the help of toluene. The residue was purified by silica gel chromatography (1:1 petroleum ether–EtOAc) to afford **15** as a foamy solid (120 mg, 81%): $[\alpha_D^{25}$ +35 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.27 (t, 1H, *J* 3.5 Hz), 5.20–5.18 (m, 2H), 5.07–4.97 (m, 3H), 4.91 (t, 1H, *J* 9.7 Hz), 4.78 (t, 1H, *J* 8.2 Hz), 4.59 (d, 1H, *J* 8.0 Hz), 4.67 (d, 1H, *J* 5.3 Hz), 4.43 (d, 1H, *J* 7.7 Hz), 4.18–4.10 (m, 2H), 3.98–3.92 (m, 2H), 3.87–3.72 (m, 4H), 3.57 (dd, 1H, *J* 10.3, 2.0 Hz), 3.23 (t, 1H, *J* 11.4 Hz), 2.92 (d, 1H, *J* 9.2 Hz), 2.80 (dd, 1H, *J* 10.1, 3.5 Hz), 2.06–1.98 (m, 24H, 8Ac), 1.11, 1.05, 0.97, 0.92, 0.89, 0.87, 0.74 (7s, 7 × 3H, 7C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.5, 171.2, 170.7 (2C), 170.6, 170.5, 170.3, 170.2, 169.7, 144.0, 123.1, 103.8, 101.8, 100.1, 98.3, 77.0, 75.5, 73.8, 72.2, 72.1, 69.9, 69.8, 69.2, 67.3, 66.9, 63.7, 62.3, 61.0, 56.0, 48.2, 47.0, 46.8, 46.4, 41.6, 41.2, 39.9, 38.1, 34.4, 33.6, 33.2, 33.0, 31.3, 30.9, 28.4, 28.1, 26.5, 24.1, 24.0, 23.5, 21.5, 21.4, 21.3, 21.2, 21.1, 18.8, 17.5, 17.4, 17.1. MALDI-TOF–MS: calcd for $C_{62}H_{90}O_{25}$: 1234.6 $[M]^+$; found, 1257.6 $[M+Na]^+$. Anal. Calcd for $C_{62}H_{90}O_{25}$: C, 60.28; H, 7.34. Found: C, 60.43; H, 7.30.

3.10. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl 3β-*O*-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-2,3-di-*O*-acetyl-β-D-xylo pyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oate (17)

To a pre-cooled stirring mixture of **15** (100 mg, 0.081 mmol), TMSOTf (8 μL, 0.04 mmol), and 4 Å molecular sieves in anhyd CH_2Cl_2 (10 mL) was added a solution of **16** (42 mg, 0.096 mmol) in CH_2Cl_2 (5 mL) under N_2 atmosphere at −78 °C. The mixture was stirred under these conditions for 30 min, then quenched by Et_3N, and filtered. The filtrate was concentrated and purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to afford **17** as a foamy solid (96 mg, 79%): $[\alpha_D^{25}$ +36 (*c* 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01 (d, 1H, *J* 1.8 Hz), 5.32 (br, 1H), 5.27 (dd, 1H, *J* 3.3, 10.2 Hz), 5.21–5.19 (m, 3H), 5.12 (t, 1H, *J* 9.9 Hz), 5.07–4.97 (m, 3H), 4.91 (t, 1H, *J* 9.8 Hz), 4.78 (t, 1H, *J* 8.0 Hz), 4.59 (d, 1H, *J* 7.9 Hz), 4.46 (d, 1H, *J* 5.2 Hz), 4.42 (d, 1H, *J* 7.7 Hz), 4.16–4.12 (m, 2H), 3.97–3.92 (m, 2H), 3.82–3.74 (m, 4H), 3.59 (dd, 1H, *J* 10.3, 2.4 Hz), 3.23 (t, 1H, *J* 11.4 Hz), 2.92 (d, 1H, *J* 9.0 Hz), 2.89 (dd, 1H, *J* 10.1, 3.6 Hz), 2.15–1.99 (m, 33H, 11 × Ac), 1.24 (d, 1H, *J* 6.1 Hz), 1.13, 1.07, 0.97, 0.95, 0.89, 0.86, 0.76 (7s, 7 × 3H, 7C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 171.2, 170.7 (2C), 170.6, 170.5, 170.3, 170.2, 169.7, 144.2, 123.4, 103.8, 101.8, 100.2, 98.3, 90.7, 77.0, 75.5, 73.8, 72.2, 72.1, 70.9, 69.9, 69.6, 69.5, 69.3, 69.1, 67.3, 66.8, 63.9, 62.3, 62.2, 56.1, 48.1, 47.8, 46.8, 46.2, 42.4, 42.2, 41.2, 39.9, 38.1, 34.3, 33.6, 33.2, 33.1, 31.3, 30.3, 28.4, 28.0, 26.4, 24.0 (2C), 23.5, 21.8, 21.5, 21.4, 21.3, 21.2, 21.1, 21.0, 18.8, 18.1, 17.7, 17.5, 17.1. MALDITOF-MS: calcd for $C_{74}H_{106}O_{32}$: 1506.7 $[M]^+$; found, 1529.7 $[M+Na]^+$. Anal. Calcd for $C_{74}H_{106}O_{32}$: C, 58.95; H, 7.09. Found: C, 59.18; H, 6.97.

3.11. α-L-Rhamnopyranosyl 3β-*O*-[α-L-arabinopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl]-2α-hydroxyolean-12-en-28-oate (1)

To a stirred solution of **17** (60 mg, 0.04 mmol) in $MeOH/CH_2Cl_2$ (v/v, 1:1, 10 mL) was added 1 M NaOMe until pH 10 was reached. The mixture was stirred at rt for 3 h at pH 9, then neutralized with Dowex-50 (H^+) ion exchange resin, filtered, and the filtrate was concentrated. The residue was purified by 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 (38 mg, 91%): $[\alpha_D^{25}$ −55 (*c* 0.4, CH_3OH); Selected ^{1}H NMR (400 MHz, CD_3OD): δ 6.01 (d, 1H, *J* 1.8 Hz, H-1^{Rha}), 5.29 (br s, 1H, H-12), 4.60 (d, 1H, *J* 7.6 Hz, H-1^{Xyl}), 4.39 (d, 1H, *J* 7.4 Hz, H-1^{Glc}), 4.24 (d, 1H, *J* 6.9 Hz, H-1^{Ara}), 3.88–3.21 (m, 21H), 2.98 (d, 1H, *J* 8.8 Hz), 2.92 (dd, 1H, *J* 10.6, 3.5 Hz, H-18), 1.24 (d, 3H, *J* 6.1 Hz, H-6^{Rha}), 1.18, 1.12, 1.02, 0.97, 0.93, 0.90, 0.80 (7s, 7 × 3H, 7C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.3, 144.1, 123.4, 104.9, 103.8, 103.0, 95.6, 94.1, 81.6, 77.7, 77.2 (2C), 75.3, 75.0, 73.3 (2C), 72.6 (2C), 71.6, 71.2, 70.6, 70.2, 68.9, 67.1, 66.5, 63.9, 61.4, 55.9, 48.8 (2C), 47.5, 46.7, 45.1, 40.9, 39.9, 37.9, 33.9, 33.2, 33.0, 32.6, 30.8, 27.4 (2C), 25.6, 23.8, 23.2, 23.1, 18.5, 17.4, 17.3, 16.8, 16.3. MALDITOF-MS: calcd for $C_{62}H_{90}O_{25}$: 1044.6 $[M]^+$; found, 1067.9 $[M+Na]^+$. Anal. Calcd for $C_{52}H_{84}O_{21}$: C, 59.75; H, 8.10. Found: C, 59.49; H, 8.19.

Acknowledgments

This work was supported in partial by NNSF of China (projects 20872172, 20732001, 20921063, and 20890112). We would like to thank Dr. Jianjun Zhang of CAU for providing compounds **9** and **16** as the starting materials.

References

1. Wang, K.-W.; Zhang, H.; Shen, L.-Q.; Wang, W. *Carbohydr. Res.* **2011**, *346*, 253–258.
2. Kalinin, V. I.; Silchenko, A. S.; Avilov, S. A.; Stonik, V. A.; Smirnov, A. V. *Phytochem. Rev.* **2005**, *4*, 221–236.
3. Itabashi, M.; Segawa, K.; Ikeda, Y.; Kondo, S.; Naganawa, H.; Koyano, T.; Umezawa, K. *Carbohydr. Res.* **1999**, *323*, 57–62.
4. Yu, B.; Zhang, Y.; Tang, P. *Eur. J. Org. Chem.* **2007**, 5145–5161.
5. Yu, B.; Sun, J. *Chem. Asian J.* **2009**, *4*, 642–654.
6. Hashimoto, G.; Nishimoto, Y. *Illustrated Cyclopedia of Brazilian Medicinal Plants*; Aboc-shya: Kanagawa, Japan, 1996. pp 734–735.
7. da Silva Rodrigues, A.; Chaves, N. S. T.; Damasceno, A. D.; Trinidade, B. R.; Martins, G. H. L.; Arantes, A. F. *Cien. Anim. Bras.* **2005**, *6*, 119–126.
8. Yokosuka, A.; Kawakami, S.; Haraguchi, M.; Mimaki, Y. *Tetrahedron* **2008**, *64*, 1474–1481.
9. Zhu, C.; Tang, P.; Yu, B. *J. Am. Chem. Soc.* **2008**, *130*, 5872–5873.
10. Levy, M.; Zehavi, U.; Naim, M. *Carbohydr. Res.* **1989**, *193*, 115–123.
11. Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhardt, R. J. *Org. Lett.* **2003**, *5*, 3627–3630.
12. Gu, G.; Du, Y.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 5497–5500.
13. Wen, X.; Sun, H.; Liu, J.; Cheng, K.; Zhang, P.; Zhang, L.; Hao, J.; Zhang, L.; Ni, P.; Zographos, S. E.; Leonidas, D. D.; Alexacou, K. M.; Gimisis, T.; Hayes, J. M.; Oikonomakos, N. G. *J. Med. Chem.* **2008**, *51*, 3540–3554.
14. Liu, M.; Fan, H.; Guo, Z.; Hui, Y. *J. Carbohydr. Chem.* **1996**, *15*, 1139–1145.
15. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353–3356.
16. Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 8165–8176.
17. Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576–6583.
18. Helm, R. F.; Ralph, J.; Anderson, L. *J. Org. Chem.* **1991**, *56*, 7015–7021.
19. Wang, P.; Li, C.; Zang, J.; Song, N.; Zhang, X.; Li, Y. *Carbohydr. Res.* **2005**, *340*, 2086–2096.
20. Paquette, L. A.; Gao, Z.; Ni, Z.; Smith, G. F. *Tetrahedron Lett.* **1997**, *38*, 1271–1274.

21. Zhang, J.; Zhu, Y.; Kong, F. *Carbohydr. Res.* **2001**, *336*, 229–235.
22. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Hirano, T.; Oka, K.; Dobashi, A.; Li, H.; Harada, N. *Tetrahedron* **1997**, *53*, 11549–11562.
23. Wu, F.; Yi, Y.; Sun, P.; Zhang, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6430–6433.
24. Zehavi, U.; Ziv-Fecht, O.; Levy, M.; Naim, M.; Evron, R.; Polacheck, I. *Carbohydr. Res.* **1993**, *244*, 161–169.
25. Kuruuzum-Uz, A.; Guvenalp, Z.; Kazaz, C.; Salih, B.; Demirezer, L. O. *Helv. Chim. Acta* **2010**, *93*, 457–465.
26. Zareen, S.; Choudhary, M. I.; Akhtar, M. N.; Ngounou, F. N. *Phytochemistry* **2008**, *69*, 2400–2405.