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Carbohydrate Research

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Xin Meng [a], Yiwu Pan [a], Tao Liu [a], Chen Luo [a], Shuli Man [a], Yongmin Zhang [a,c], Yan Zhang [b, *]

[a] *Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, China International Science and Technology Cooperation Base of Food Nutrition/Safety and Medicinal Chemistry, Tianjin 300457, PR China*
[b] *Department of Pharmacy, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, PR China*
[c] *Sorbonne Université, Institut Parisien de Chimie Moléculaire, UMR CNRS 8232, 4 place Jussieu, 75005, Paris, France*

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ABSTRACT

Diosgenyl saponins, as a type of natural products derived from plants, are the main active component of traditional chinese medicine. Inspiringly, a large number of natural diosgensyl saponins have been shown to exert excellent toxicity to hepatocellular cancer (HCC) cells. In order to better understand the relationship between the structures and their biological effects, a group of diosgenyl saponins (**1-4** as natural products and **5** and **6** as their analogues) were efficiently synthesized. The cytotoxic activity of these compounds was evaluated on human hepatocellular carcinoma (HepG2) cells. Structure–activity relationship studies showed that the pentasaccharide or hexasaccharide saponin analogues were relatively less active than their corresponding disaccharide analogue or dioscin. The extension of 4-branched rhamnose moiety on these saponin does not exhibit significant effect on their cytotoxic activity, which disclosed that a certain number and the linkage mode of rhamnose moieties could influence the cytotoxicity of steroid saponins on HepG2 cells.

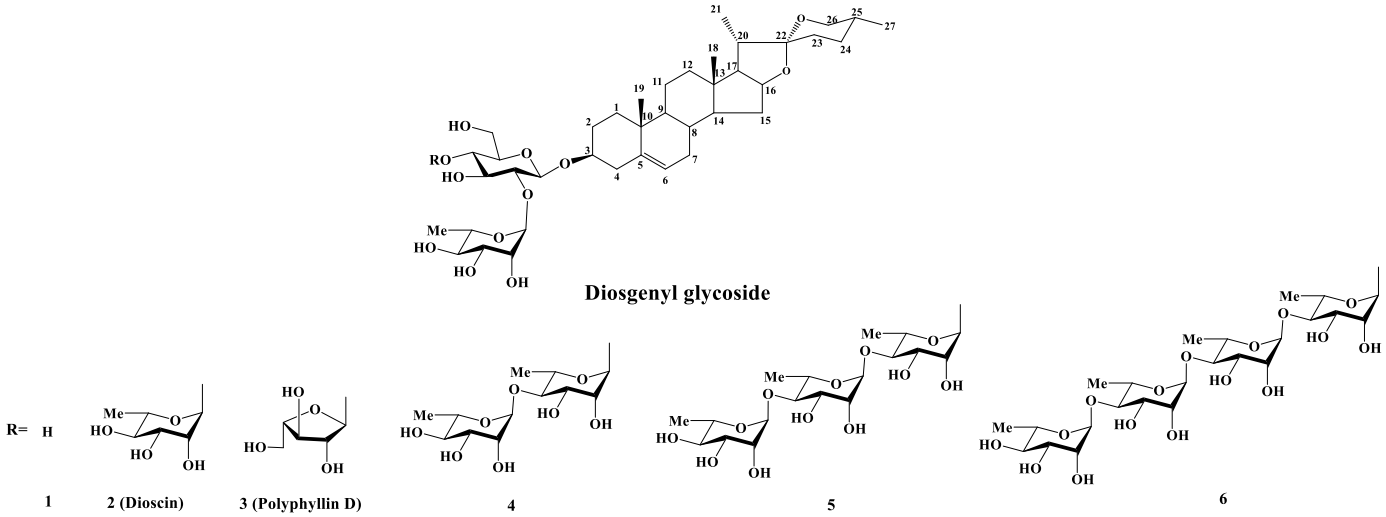
1. Introduction

Hepatocellular cancer (HCC), the most common liver cancer, is rising at an alarming rate worldwide and it leads to advanced liver diseases. Many reports suggested that diosgenin (DSG) and diosgenyl saponins imparted anti-cancer effects on HCC cells [1-4]. However, poor pharmacokinetic profile, low aqueous solubility, and instability in different physiological conditions limit the usage of DSG for clinical applications. A modification at the C3 position of DSG changes its physiochemical characteristics drastically and improves the adsorption, distribution, metabolism, elimination, and biological activities of DSG [5-7].

The typical structure of the glycan in diosgenyl saponins is one with a β-D-glucopyranoside as the first sugar attached to diosgenin, which in turn has an α-L-rhamnopyranose substituted at 2-OH and another sugar chain at 4-OH. The structure diversity of the steroidal saponins mainly lay on the carbohydrate moieties present on it, which effected their activities significantly [8]. In the last two decades, an increasing number of natural and non-natural steroidal saponins have been synthesized and evaluated for their anticancer activity [9]. Many studies have disclosed that DSG glycosides were better apoptosis-inducers than DSG and the structure of the sugar residues, especially for the 2-OH and 4-OH branches elongated from the glucose and rhamnose residues play crucial roles in triggering cell death by apoptosis [10-14]. However, only the activity of diosgenyl saponins containing shorter glycans than trisaccharide have been relatively extensive studied [15,16]. While, the structure-activity relations of diosgenyl saponins containing longer 2, 4-branched oligosaccharides moiety have never been explored and complex diosgenyl saponins with longer carbohydrate residues were rarely synthesized [17].

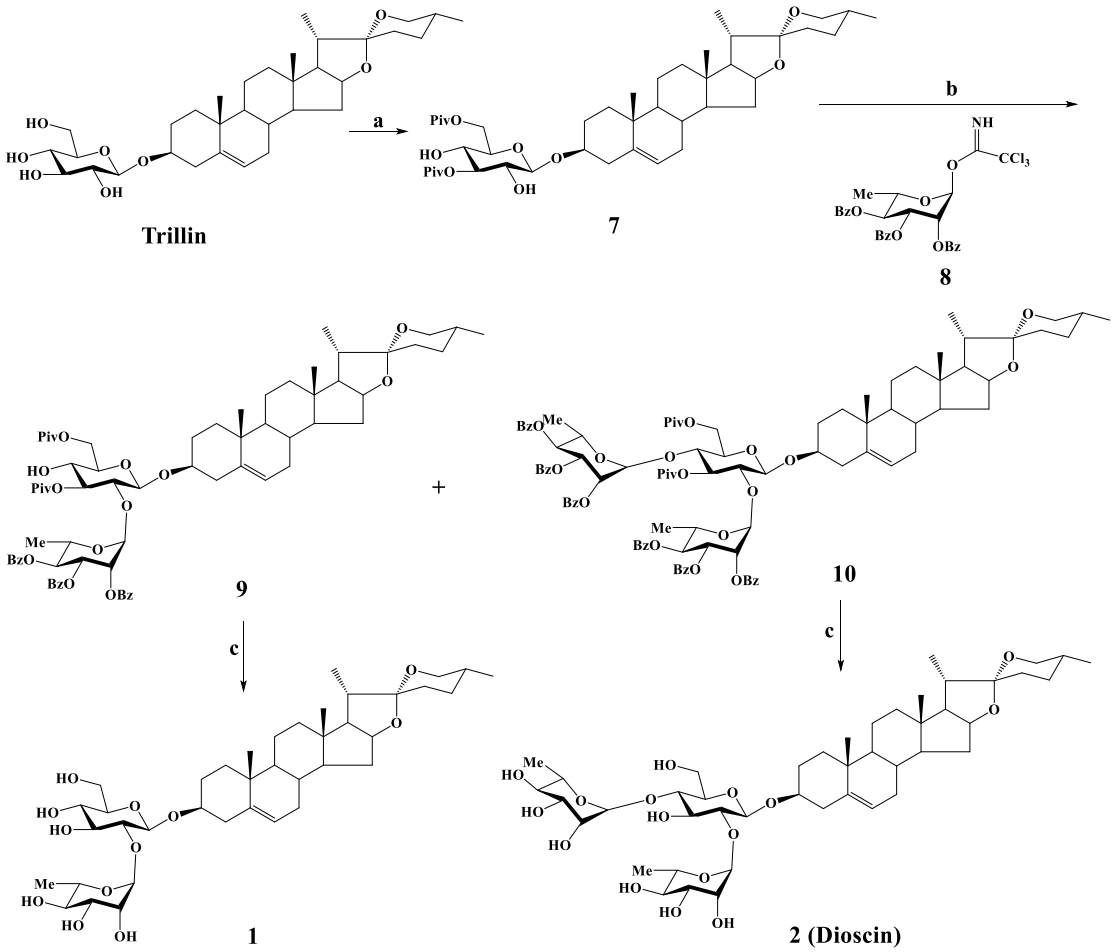
Our research group has reported the activity of some steroidal glycosides, in which, their structure-activity relationships and roles as apoptosis or necrosis inducers have been established [3, 18-21]. Specially, these bioactive saponins are comprised of a 2,4-branched oligosaccharide moiety. Because of their biological funtions and also their unique 2,4-dibranched chain structures, the efficient synthesis of these diosgenyl glycosides deserves extensive exploration. As part of the ongoing efforts in discovering new bioactive compounds, the present work is concerned with the structure–activity relationship upon the sugar chains of diosgenyl glycosides. A series of diosgenyl glycosides (Fig. 1), **1** (diosgenyl O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside); **2** Dioscin (diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-α-D-glucopyranoside), **3** polyphyllin D (diosgenyl α-L-rhamno-pyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-β-D-glucopyranoside), **4** (diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside), **5** (diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)]-β-D-

* Corresponding author. E-mail: zhangyansdu@email.sdu.edu.cn (Y. Zhang)



1 Rhaα-(1→2)-Gluβ→Diosgenin
2 Rhaα-(1→4)-[Rhaα-(1→2)]-Gluβ→Diosgenin (Dioscin)
3 Araα-(1→4)-[Rhaα-(1→2)]-Gluβ→Diosgenin (Polyphyllin D)
4 Rhaα-(1→4)-Rhaα-(1→4)-[Rhaα-(1→2)]-Gluβ→Diosgenin
5 Rhaα-(1→4)-Rhaα-(1→4)-Rhaα-(1→4)-[Rhaα-(1→2)]-Gluβ→Diosgenin
6 Rhaα-(1→4)-Rhaα-(1→4)-Rhaα-(1→4)-Rhaα-(1→4)-[Rhaα-(1→2)]-Gluβ→Diosgenin

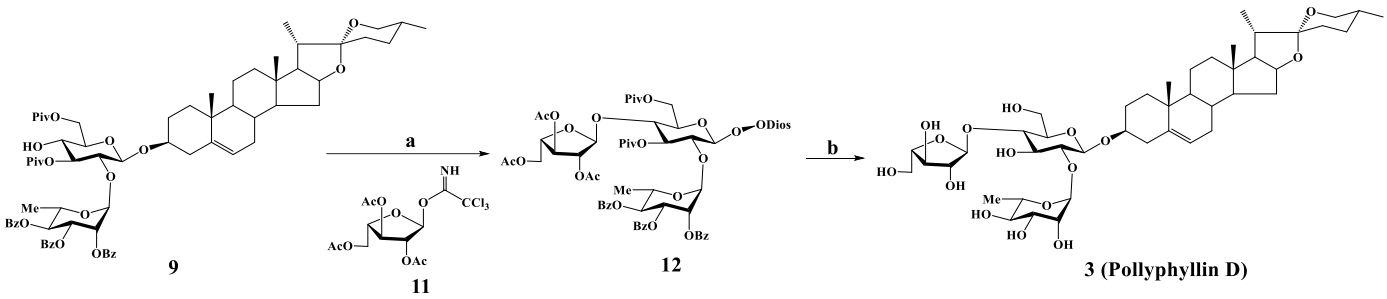
Fig. 1. Diosgenyl saponins and their analogs.



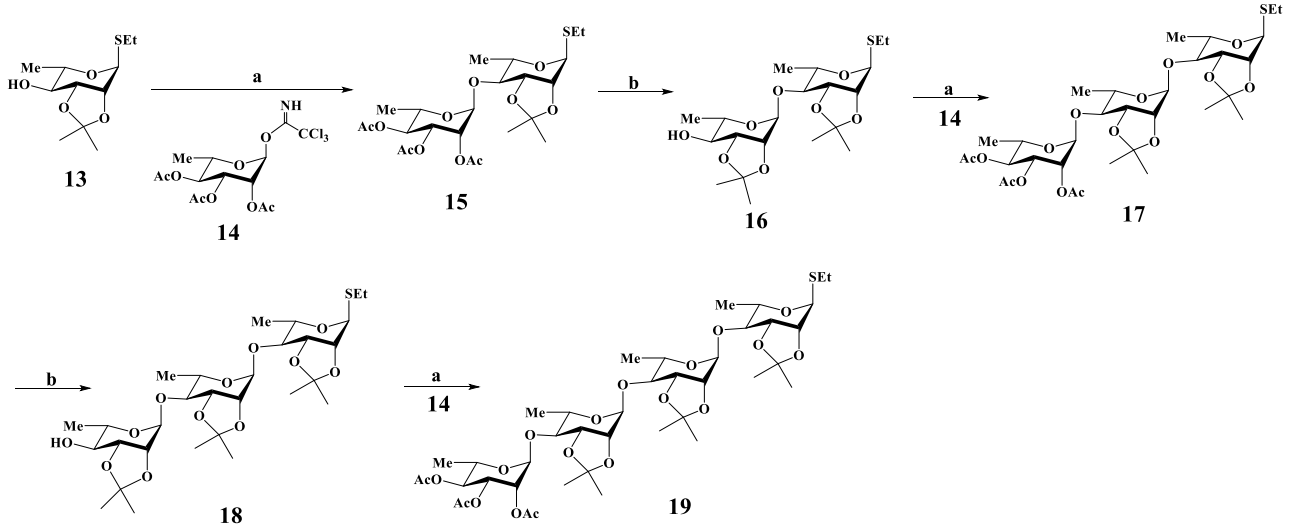
Scheme 1. Synthesis of diosgenyl glycosides **1** and **2** (dioscin). Reagents and conditions—a: PivCl, CH_2Cl_2/Pyridine (v/v= 3:1), - 10 ℃ to 0 ℃ , 80%; b: **8** (1.2 equiv), 4Å MS, TMSOTf (0.2 equiv), - 60 ℃; 50% for **9**, 32% for **10**; c: THF/MeOH/H_2O (v/v/v=4:4:1), NaOH, 45 ℃, 80% for **1**, 85% for **2** (Dioscin).

glucopyranoside), **6** (diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside) were designed and synthesized. Among them, **1**-**4** were natural products and **5** and **6** were first synthesized. Furthermore, the inhibitory effect of the obtained target molecules against HepG2 cell line (hepatocellular carcinoma cell line) were also evaluated, and the preliminary structure-activity relationship was elucidated.

2. Results and discussion



Scheme 2 Synthesis of Polyphyllin D. Reagents and conditions- a: **11** (2.5 equiv), $BF_3{\cdot}Et_2O$ (0.7 equiv), 4Å MS, CH_2Cl_2, 0℃, 93%. b: THF/MeOH/H_2O (v/v/v= 4:4:1), NaOH, 45 ℃, 90%.

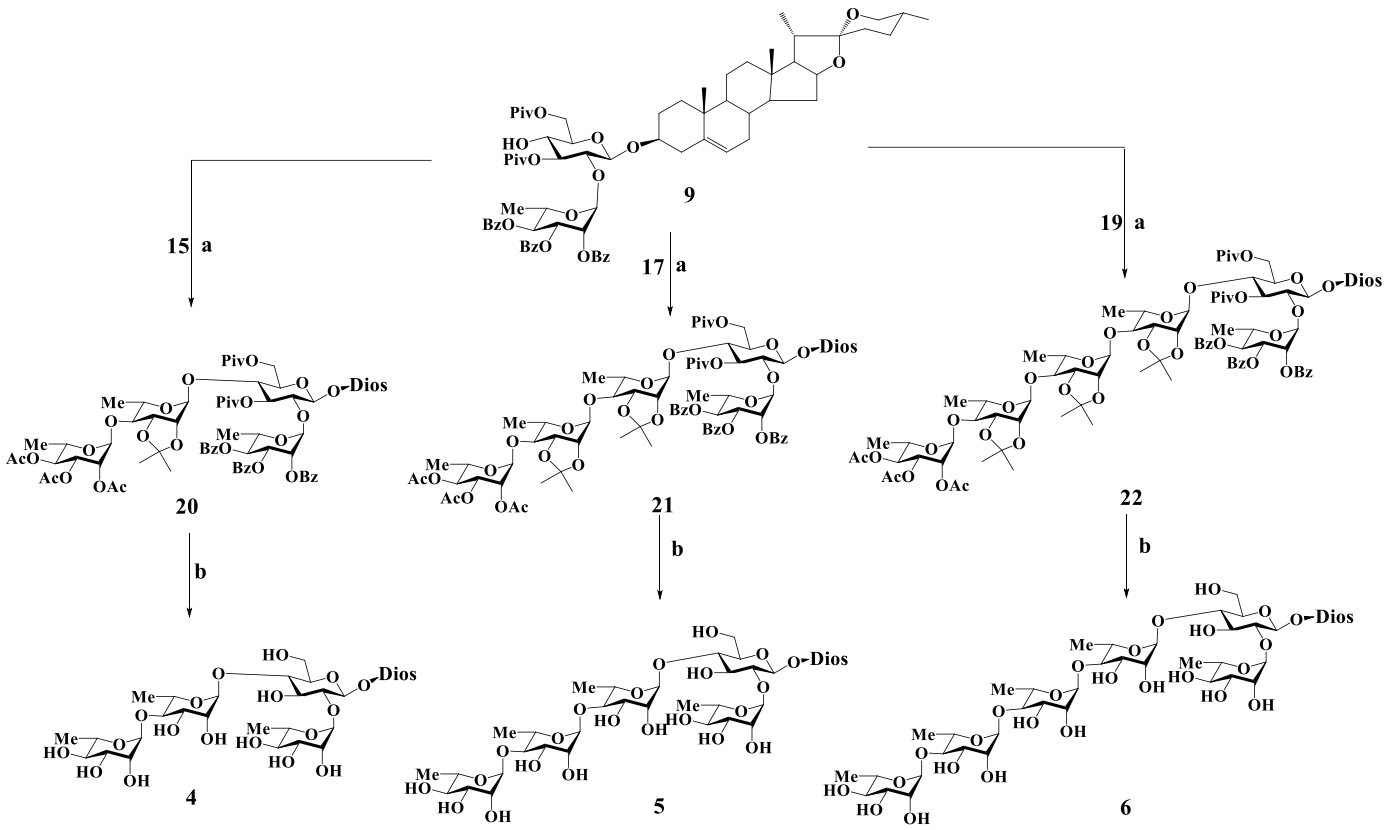


Scheme 3. Synthesis of donors **15**, **17**, **19**. Reagents and conditions- a: TMSOTf, CH_2Cl_2, 4Å MS, -78℃; 92% for **15**, 82% for **17** and 83% for **19**. b: (i) MeONa/MeOH; (ii) $TsOH{\cdot}H_2O$, 2,2-Dimethoxypropane, -10 ℃, 90% over two steps for **16** and 88% for **18**.

Before synthesis, we envisioned that producing two glycosylation products should be more feasible than producing only monoglycosylated products and should facilitate the final purification processes. So, we explored this idea in the preparation of the intermediate **9** and **10**. Compound **7**, containing unprotected 2,4-hydroxyl groups, was prepared from commercially available trillin (diosgenyl α-D-glucopyranoside) according to previous report [22]. As shown in Scheme 1, the synthesis was started from trillin, in the absence of an adjacent axial function, the secondary 3-OH of glucosyl may be more reactive than the secondary 2-OH toward pivaloylation. High selectivity was observed on the pivaloylation of trillin under pivaloyl chloride (PivCl) in CH_2Cl_2/pyridine (3:1) solution affording the 3,6-di-Piv-protected diol **7** in 80% yield [23]. Glycosylation of diol **7** with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate **8** [24] (1.2 equiv, -60 ℃) under the promotion of trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.2 equiv) gave the 2-*O*-glycosylated product **9** and 2,4-di-*O*-glycosylated product **10** in 50% and 32% yields, respectively. The presence of an acyl group at the 2-OH position of a sugar donor locks the newly formed glycosidic linkage in the 1,2-trans configuration [24]. Removal of all the acyl protecting groups of **9** (benzoyl and pivaloyl groups) under NaOH in a solvent mixture of THF/MeOH/H_2O (v/v/v= 4:4:1) furnished the naturally existing saponin **1** in 90% yield. Deprotection of **10** under similar conditions afforded the desired **2** (dioscin) in good yield (88%). The physical data of **2** were identical with those reported [26].

The synthesis of fully protected target molecule polyphyllin D weas commenced with glycosylation between **9** and 2,3,5-tri-*O*-acytyl-α-L-arabinofuranosyl trichloroacetimidate **11** [26]. Under the effect of Lewis-acid $BF_3{\cdot}OEt_2$, the conjugation of **9** and **11** proceeded fluently to afford the fully protected trisaccharide glycoside **12** (90%). Removal of all the acyl protecting groups of **12** (acetyl, benzoyl and pivaloyl groups) under NaOH in a solvent mixture of THF/MeOH/H_2O (v/v/v= 4:4:1) afforded polyphyllin D in 90% yield, whose data was in good accordance with those reported (Scheme 2) [26]. It is worth mentioning, when we replaced $BF_3{\cdot}OEt_2$ with TMSOTf [27] as promoter, a mixture of α (30%) and β (40%) 4-OH glycosylation products were afforded (data not shown).

As shown in Scheme 3, we used the ethylthiogroup as the rhamnose C-1 OH's protection group for easier operation. After protecting the rhamnose C-2 and C-3 hydroxyl with 2,2-dimethoxypropane under the promotion of TsOH, we got the acceptor **13**. Then the glycosylation of ethyl 2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside **13** with 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl trichloroacetimidate **14** (1.2 equiv) under the promotion of TMSOTf (0.2 equiv) at -78 ℃ provided the ethylthio disaccharide **15** (92%). It is worth mentioning that the glycosylation shoud be performed at -78℃. Increased glycosylation temperature leads to intermolecular ethylthio-group transfer by-product [17]. The newly formed α-rhamnopyranosyl linkage in **15** was confirmed by the anomeric signals in its ^1H and ^{13}C NMR spectra: δ 5.26 ppm (d, J = 1.2 Hz, H-1^{II}) and δ 95.85 ppm (C-1^{II}). Then, the acetyl group of **15** was removed under alkaline condition, and then isopropylidene group was used to protect vicinal *syn*-3,4-diol was further blocked by isopropylidenyl group to furnish **16** (90%). The following steps



Scheme 4. Synthesis of diosgenyl glycosides **4**, **5** and **6**. Reagents and conditions- a: donor **15, 17 or 19**, NIS, AgOTf, 4Å MS, -10 °C; 87% for **20**, 51% for **21** and 60% for **22**; b: 80% AcOH, 80 °C, 5 h; THF/MeOH/H_2O(v/v/v=1:1:1), NaOH, 45°C, 18 h, 93% over two steps for **4**, 90% for **5**, 88% for **6**.

Table 1. Cytotoxicities (IC_{50}) of Trillin, Dioscin, Polyphyllin D and their analogs against HepG2 Cells[a]

Compound	Trillin	1	2 (Dioscin)	3 (Pollyphyllin D)	4	5	6
IC_{50} (μM)[b]	108.7 ± 8.9	2.92 ± 0.19	3.09 ± 0.12	12.17 ± 1.11	6.33 ± 0.39	22.84 ± 3.61	13.42 ± 0.50

[a] HepG2 (liver hepatocellular carcinoma)

[b] IC_{50} values (50% inhibition concentration) represent the means of three independent experiments with SD.

for the preparation of donors **17** and **19** followed the same approach as that used for the synthesis of **15** and **16**. Glycosylation of the acceptor **16** with the L-rhamnopyranosyl imidate **14** in the presence of TMSOTf as the catalyst at -78 °C afforded the coupling product **17** in excellent yield (82%). The newly formed α-rhamnopyranosyl linkage in **17** was confirmed by the anomeric signals in its ^{1}H and ^{13}C NMR spectra: δ 5.35 ppm (br s, H-1^{III}) and δ 95.38 ppm (C-1^{III}). NaOMe mediated saponification delivered triol intermediate and then regioselective protection of the vicinal *syn*-3,4-diol of the triol intermediate with an isopropylidenyl group to afford acceptor **18** (88%), which was primed for subsequent sugar chain extension. Thus, upon coupled with rhamnopyranosyl trichloroacetimidate **14**, the trisaccharide glycoside acceptor **18** was transformed to tetrasaccharide glycoside **19** efficiently (83%) [28].

With donors and acceptors in hand, we sought to prepare saponin **4**–**6** followed the approach as shown in Scheme 4. Coupling reaction between saponin acceptor **9** and thiodisaccharide **15** was first investigated. Glycosylation of **9** with the ethylthio donor **15** (2.0 equiv) under the action of NIS-AgOTf gave the expected 2,4-di-O-glycosylated product **20** in satisfactory yield (87%). With the successful synthesis of protected saponin **20,** we explored to apply the strategy for synthesizing **21** and **22**. Under similar conditions, pentasaccharide saponin **21** was obtained in 51% yield by coupling of thiotrisaccharide **17** with **9**, whereas 20% of the acceptor **9** was recovered. Glycosylation of **9** with thiotetraaccharide **19** (1.2 equiv) under the same condition provided the desired **22** in lower 60% yield (compared to the yield of 87% for **20**). Treatment of fully protected steroidal saponins **20**-**22** with 80% AcOH to cleave isopropylidene groups, following NaOH to remove the acyl protecting groups (acetyl, benzoyl and pivaloyl), afforded the desired saponins **4**–**6** in very good yields (88–93%). Saponin **4** gave identical analytical data with those reported [28]. The Rha-(1→4)-Glu linkages, constructed by using donors (**17** and **19**) without a neighboring participating group, were confirmed to be α configurations by measuring the $J_{C1–H1}$ values (169.7 and 169.5 Hz, respectively) of corresponding rhamnose residues in saponins **5** and **6**.

To date, limited information on the structure activity relationship (SAR) of diosgenyl saponins and their cytotoxicity is available [29]. Although both the aglycone and the carbohydrate moieties are required for the exhibition of their antitumor activity, the exact role of the carbohydrate moiety remains unclear. Thus, with target six compounds in hand, the cytotoxicity of the saponins was evaluated [24, 30-33]. The group of steroidal saponins exhibited a marked structure-dependent growth inhibitory activity against the human hepatocellular carcinoma (HepG2) cells (Table1), which supported that the saccharide group increased the cytotoxicity of trillin, especially for rhamnose. Moderate inhibitory activity was observed for most compounds against HepG2 cell lines, with

IC_{50} values in the μM range, varying from 2.92 μM for compound **1** to greater than 20 μM for compound **5**. Diosgenyl α-L-rhamnopyranosyl (1→2)-β-D-glucoside **1** showed potent anticancer activities to chosen cell lines, which indicated that the structural modification at the 2'-OH position of the glucose residue appears to affect the potency of its anticancer activity. Additionally, with an α-L-rhamnopyranose substituted at 4'-OH (dioscin), the cytotoxicity activities did not change greatly compared to **1**. However, substituting α-L-rhamnopyranose residue at 4'-OH position with α-L-arabinofuranose (polyphyllin D), the cytotoxicity activities reduced to 12.17 μM, which indicated the importance of rhamnose moiety. Interestingly, extending the sugar chain at 4'-OH with more rhamnose moieties, the diosgenyl pentasaccharide **5** and hexasaccharide **6** showed cytotoxicity activities of IC_{50} over 10 μmol/L. This may indicate that extending 4'-OH branched with rhamnose moieties do not result in increased anticancer activity toward HepG2 cell. In addition, effects of compound **1** and **2** on the growth of human normal liver cells L-02 was further investigated and the IC_{50} values of compound **1** and **2** was 49.23±0.06 and 111.1±0.19 μM, respectively. The MTT results indicated compound **1** and **2** showed no obvious cytotoxicities to human normal liver cells L-02. The action mechanism of the anticancer activity of diosgenyl saponins will be explored in further work.

3. Conclusions

In summary, a series of diosgenyl glycosides with 2,4-branched oligosaccharide moieties were synthesized in an efficient and practical way. The anticancer activities *in vitro* of synthesized saponins were evaluated by MTT assay. Moderate cytotoxic activity is found for analogs against HepG2 cells. The result also disclosed that the number and linkage mode of rhamnose moieties have an important effect on the antitumor activities of diosgenyl glycosides. Efforts are currently under way to examine the intercellular target and actions of these saponins by *in vitro* and *in vivo* studies.

4. Experimental

4.1 Materials and methods

^1H, ^{13}C and nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III (400 MHz) spectrometer using $CDCl_3$, MeOD, DMSO-d6 as the solvent with TMS as an internal standard. Chemical shifts were reported as δ (ppm) and spin-spin coupling constants as *J* (Hz) values. High resolution ESI mass spectra were obtained at a hybrid IT-TOF mass spectrometer (Shimadzu LCMS-IT TOF, Kyoto, Japan). The compounds were stained with 5% H_2SO_4 in ethanol and detection with UV light was employed when possible. Flash column chromatography was performed on silica gel 300~400 mesh. All of the starting materials are commercially available and were used without further purification.

4.2. Synthesis

4.2.1 Diosgenyl 3, 6-di-O-pivaloyl-β-D-glucopyranoside (7)

To a solution of trillin (diosgenyl β-D-glucopyranoside) (1 g, 1.73 mmol) in CH_2Cl_2-pyridine (3:1 v/v, 8 mL) at -10 °C was slowly added pivaloyl chloride (0.85 mL, 6.91 mmol). The reaction was monitored by TLC, and the temperature was allowed to warm to 0 °C. When most of the mono-pivaloylated product disappeared, 1 mL MeOH was added to quench the reaction. The solvent was removed in vacuo and the residue was diluted with CH_2Cl_2 (20 mL) and then washed with 1 M HCl solution, saturated aqueous $NaHCO_3$ and brine, respectively. The organic layer was dried over Na_2SO_4 and concentrated. The residue was subjected to flash silica gel column chromatography (3:1 petroleum ether-EtOAc) to give **7** (1.03 g, 80%) as a white foam. R_f = 0.50 (PE: EtOAc, 3:1); ^1H NMR (400 MHz, $CDCl_3$) δ 5.35 (d, 1H, *J* = 5.0 Hz), 4.85 (t, 1H, *J* = 9.2 Hz), 4.45-4.39 (m, 3H), 4.25 (dd, 1H, *J* =11.8, 6.4 Hz), 3.60-3.40 (m, 5H), 3.37 (t, 1H, *J* = 10.8 Hz), 3.05 (d, 1H, *J* =4.8), 2.42-2.30 (m, 2H), 2.29-2.20 (m, 1H), 2.15-1.90 (m, 3H), 1.89-1.80 (q, 2H), 1.79-1.70 (q, 4H), 1.69-1.55 (m, 7H), 1.54-1.45 (m, 5H), 1.26-1.13 (m, 4H), 1.12-0.85 (m, 9H), 0.80-0.70 (d, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.24, 178.60, 140.15, 130.88, 128.81, 121.91, 109.27, 101.22, 80.78, 79.57, 77.93, 74.19, 72.05, 70.06, 66.83, 65.54, 63.59, 62.07, 56.44, 50.03, 41.58, 40.24, 39.72, 39.03, 38.82, 38.80, 37.15, 36.82, 32.03, 31.82, 31.38, 30.54, 30.27, 29.63, 28.77, 26.86, 20.81, 19.33, 19.15, 17.11, 16.25, 14.50. HRMS m/z calcd for $C_{43}H_{69}O_{10}$ $[M+H]^+$: 745.4891, found: 745.4848.

4.2.2 Diosgenyl 2,3,4-tri-O-benzoyl-α–L-rhamnopyranosyl-(1→2)-3,6-di-O-pivaloyl-β-D-glucopyranoside (9) and diosgenyl 2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→4)-[2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-O-pivaloyl-β-D-glucopyranoside (10)

To a suspension of diol **7** (500 mg, 0.67 mmol), 2,3,4-tri-O-benzoyl-L-rhamnopyranosyl trichloroacetimidate **8** (500 mg, 0.81 mmol), and 4 Å MS (1.0 g) in dry CH_2Cl_2 (15 mL) at -60 °C, was added TMSOTf (24 μL, 0.13 mmol). After stirring 2 h at -60 °C, the reaction mixture was quenched with Et_3N (0.1 mL) and then filtered and concentrated.

Purification by silica gel column chromatography (petroleum ether: EtOAc, 4:1) gave **9** as a white foam (400 mg, 50 %). $[\alpha]_D$ +40° (c 1.01, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.6 Hz, 2H), 7.91 (d, *J* = 7.6 Hz, 2H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.26 – 7.22 (m, 2H), 5.76 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.63 (t, *J* = 10.0 Hz, 2H), 5.43 (d, *J* = 4.4 Hz, 1H), 5.23 (s, 1H), 5.18 (t, *J* = 9.4 Hz, 1H), 4.79 (dq, *J* = 12.2, 6.0 Hz, 1H), 4.69 (d, *J* = 7.8 Hz, 1H), 4.48 – 4.39 (m, 3H), 4.26 (dd, *J* = 11.8, 6.8 Hz, 1H), 3.84 (t, *J* = 8.4 Hz, 1H), 3.65 (ddd, *J* = 17.0, 10.4, 4.6 Hz, 2H), 3.50 – 3.35 (m, 4H), 3.06 (d, *J* = 6.0 Hz, 1H), 2.60 – 2.52 (m, 1H), 2.42 (t, *J* = 12.6 Hz, 1H), 2.05 – 1.98 (m, 3H), 1.86 – 1.73 (m, 5H), 1.62 (d, *J* = 13.8 Hz, 6H), 1.35 (d, *J* = 6.2 Hz, 3H), 1.22 (t, *J* = 7.8 Hz, 15H), 1.17 (s, 8H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.93 (s, 3H), 0.79 (d, *J* = 6.2 Hz, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.90, 178.53, 167.66, 165.62, 165.39, 165.15, 139.99, 133.39, 133.19, 132.99, 132.26, 130.86, 129.78, 129.72, 129.62, 129.15, 128.79, 128.53, 128.30, 128.18, 122.10, 109.23, 101.19, 99.96, 97.61, 80.75, 79.73, 78.89, 74.56, 74.08, 71.74, 71.01, 70.34, 69.85, 66.80, 66.70, 65.71, 63.59, 62.07, 56.37, 49.98, 41.57, 40.22, 39.65, 38.98, 38.81, 38.78, 37.11, 36.78, 32.08, 31.85, 31.45, 31.36, 30.51, 30.24, 29.86, 29.63, 28.76, 27.13, 26.85, 20.75, 19.19, 19.13, 17.35, 17.09, 16.23, 14.49, 13.67. HRMS m/z calcd for $C_{70}H_{90}O_{17}Na$ $[M+Na]^+$: 1225.6070, found: 1225.6026.

Purification by silica gel column chromatography (petroleum ether: EtOAc, 6:1) gave **10** as a white foam (360 mg, 32 %). $[\alpha]_D$ +64° (c 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05-7.95 (m, 4H), 7.90 (s, 1H), 7.89 (s, 1H), 7.86 (s, 1H), 7.84 (s, 1H), 7.73 (d, *J* = 7.8 Hz, 2H), 7.64 (t, *J* = 6.6 Hz, 1H), 7.52 (dd, *J* = 9.2, 4.8 Hz, 2H), 7.47 – 7.07 (m, 17H), 5.74 – 5.67 (m, 1H),

5.67 – 5.62 (m, 1H), 5.62 – 5.57 (m, 1H), 5.54 (dd, *J* = 16.4, 6.8 Hz, 1H), 5.46 (s, 1H), 5.42 (d, *J* = 7.7 Hz, 1H), 5.38 (d, *J* = 5.2 Hz, 1H), 5.22 (s, 1H), 5.15 (s, 1H), 5.09 (s, 1H), 4.71 (t, *J* = 8.4 Hz, 1H), 4.68 – 4.61 (m, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.34 (dt, *J* = 21.0, 6.2 Hz, 2H), 4.26 – 4.20 (m, 1H), 3.92 – 3.83 (m, 1H), 3.74 (dd, *J* = 14.6, 7.2 Hz, 1H), 3.67 – 3.56 (m, 1H), 3.40 (t, *J* = 10.4 Hz, 1H), 3.31 (t, *J* = 10.8 Hz, 1H), 2.52 (d, *J* = 10.4 Hz, 1H), 2.38 – 2.28 (m, 1H), 1.99 – 1.91 (m, 2H), 1.84 – 1.33 (m, 15H), 1.32 – 1.21 (m, 8H), 1.20 – 1.12 (m, 12H), 1.11 – 0.97 (m, 9H), 0.95 – 0.83 (m, 7H), 0.79 – 0.65 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.87, 176.63, 165.77, 165.67, 165.40, 165.34, 165.18, 165.06, 140.08, 133.39, 133.30, 133.27, 133.21, 133.16, 133.03, 132.95, 132.79, 130.88, 129.89, 129.82, 129.77, 129.73, 129.67, 129.61, 129.41, 129.35, 129.30, 129.25, 129.22, 128.81, 128.54, 128.51, 128.39, 128.33, 128.30, 128.22, 128.17, 128.06, 109.26, 99.44, 97.36, 80.79, 79.26, 76.32, 75.59, 72.53, 71.90, 71.84, 71.33, 70.95, 70.46, 69.88, 69.72, 69.63, 68.27, 66.82, 65.54, 62.09, 56.41, 50.01, 41.59, 40.25, 39.68, 38.87, 38.60, 37.16, 36.82, 32.12, 31.87, 31.47, 31.39, 30.54, 30.27, 29.83, 28.78, 27.18, 26.93, 20.77, 19.23, 19.15, 17.73, 17.48, 17.11, 16.25, 14.50, 13.69. HRMS m/z calcd for $C_{97}H_{113}O_{24}$ $[M+H]^+$: 1661.7622, found: 1661.7627.

4.2.3 Diosgenyl α-L-rhamnopyranosyl-(1→2)- β-D-glucopyranoside (1)

Compound **9** (50 mg) was dissolved in a solution of CH_3OH-THF-H_2O (9 mL, v/v/v=4:4:1), then NaOH was added to get pH 11.0. The solution, kept overnight at room temperature, was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. Chromatography of the residue on a silica gel column (CH_2Cl_2: CH_3OH 10:1-3:1) gave compound **1** as white solid (24 mg, 80%). $[\alpha]_D$ -96° (*c* 1.0, pyridine); ^{1}H NMR (400 MHz, C_5D_5N) δ 6.40 (s, 1H), 5.31 (d, *J* = 3.8 Hz, 1H), 5.07 – 4.96 (m, 2H), 4.86 (s, 1H), 4.65 (dd, *J* = 9.2, 3.0 Hz, 1H), 4.59 – 4.49 (m, 2H), 4.46 – 4.34 (m, 2H), 4.28 (dd, *J* = 10.4, 7.4 Hz, 2H), 4.18 (t, *J* = 8.8 Hz, 1H), 4.01 – 3.84 (m, 2H), 3.64 – 3.45 (m, 2H), 2.86-2.70 (m, 2H), 2.15 (d, *J* = 11.2 Hz, 1H), 2.09 – 2.00 (m, 1H), 1.98 – 1.83 (m, 3H), 1.78 (d, *J* = 6.2 Hz, 3H), 1.74 – 1.67 (m, 3H), 1.61 – 1.52 (m, 4H), 1.49 – 1.40 (m, 3H), 1.26 (t, *J* = 7.4 Hz, 9H), 1.15 (d, *J* = 7.2 Hz, 3H), 1.07 (s, 4H), 1.01-0.88 (m, 2H), 0.85 (s, 3H), 0.70 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, C_5D_5N) δ 141.24, 122.12, 109.64, 102.33, 100.73, 81.50, 79.98, 78.65, 78.30, 74.43, 73.19, 72.96, 72.15, 69.85, 67.25, 63.28, 62.99, 57.02, 50.66, 46.15, 42.36, 40.85, 40.24, 39.37, 37.90, 37.53, 32.70, 32.22, 32.08, 30.99, 30.58, 29.66, 21.48, 19.80, 19.07, 17.71, 16.72, 15.43, 8.98. HRMS m/z calcd for $C_{39}H_{62}O_{12}Na$ $[M+Na]^+$: 745.4133, found: 745.4179.

4.2.4 Diosgenyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (2)

Carried out using same procedure as for **1** to give **2** (dioscin) (85% yield) as a white solid. $[\alpha]_D$ +102° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, C_5D_5N) δ 6.41 (s, 1H), 5.87 (s, 1H), 5.31 (d, *J* = 4.4 Hz, 1H), 5.03-4.91 (m, 3H), 4.89 (d, *J* = 2.0 Hz, 1H), 4.72 (s, 1H), 4.64 (dd, *J* = 9.2, 3.2 Hz, 2H), 4.59-4.52 (m, 2H), 4.45-4.34 (m, 3H), 4.25-4.17 (m, 3H), 4.08 (dd, *J* = 12.0, 2.8 Hz, 1H), 3.92-3.80 (m, 1H), 3.62 (t, *J* = 9.6 Hz, 1H), 3.51 (t, *J* = 10.0 Hz, 1H), 2.86-2.66 (m, 2H), 2.10-2.00 (m, 2H), 1.96 (t, *J* = 6.8 Hz, 1H), 1.91-1.80 (m, 3H), 1.79-1.74 (d, *J* = 6.0 Hz, 3H), 1.73-1.66 (m, 3H), 1.62 (d, *J* = 6.4 Hz, 3H), 1.59 (d, *J* = 4.2 Hz, 3H), 1.52 (s, 1H), 1.48-1.41 (m, 3H), 1.26 (t, *J* = 7.2 Hz, 8H), 1.15 (d, *J* = 7.2 Hz, 3H), 1.06 (s, 3H), 1.00-0.89 (m, 2H), 0.84 (s, 3H), 0.70 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.18, 122.17, 109.65, 103.16, 102.22, 100.65, 81.49, 78.89, 78.47, 78.26, 78.05, 77.31, 74.35, 74.20, 73.15, 73.08, 72.90, 70.75, 69.86, 67.25, 63.28, 61.59, 57.02, 50.68, 46.18, 42.36, 40.85, 40.24, 39.35, 37.88, 32.70, 32.61, 32.22, 32.07, 30.99, 30.54, 29.66, 21.49, 19.79, 19.02, 18.88, 17.72, 16.73, 15.43, 9.02. HRMS m/z calcd for $C_{45}H_{72}O_{16}Na$ $[M+Na]^+$: 891.4718, found: 891.4738.

4.2.5 Diosgenyl 2,4,5-tri-O-acetyl-α-L-arabinofuranosyl-(1→4)-[2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-O-pivaloyl-β-D-glucopyranoside (12)

To a suspension of 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl-trichloroacetimidate **11** (0.14 g, 0.33 mmol), **9** (0.16 g, 0.13 mmol) and 4 Å MS (0.2 g) in dry CH_2Cl_2 (5 mL) at 0°C, was slowly added $BF_3{\cdot}Et_2O$ (12 μL, 0.1 mmol). After being stirred for 2 h, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. Chromatography of the residue on a silica gel column (5:1 petroleum ether– EtOAc) afforded the compound **12** (180 mg, 93%) as a white amorphous solid. $[\alpha]_D$ +15.5° (*c* 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.2 Hz, 2H), 7.92 (t, *J* = 9.6 Hz, 2H), 7.78 (dd, *J* = 9.8, 4.3 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.53 – 7.45 (m, 3H), 7.41 – 7.38 (m, 1H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.23 (t, *J* = 7.8 Hz, 2H), 5.79 – 5.70 (m, 1H), 5.64 – 5.56 (m, 2H), 5.42 (t, *J* = 8.5 Hz, 2H), 5.16 (d, *J* = 5.8 Hz, 2H), 5.02 (d, *J* = 1.3 Hz, 1H), 4.98 – 4.95 (m, 1H), 4.68 (d, *J* = 7.6 Hz, 1H), 4.54 (d, *J* = 10.1 Hz, 1H), 4.45 – 4.40 (m, 1H), 4.33 – 4.27 (m, 2H), 4.19 – 4.14 (m, 2H), 3.81 (q, *J* = 7.7 Hz, 2H), 3.66 (dt, *J* = 9.3, 6.6 Hz, 2H), 3.47 (d, *J* = 13.2 Hz, 1H), 3.37 (t, *J* = 10.9 Hz, 1H), 2.55 (dd, *J* = 13.0, 2.8 Hz, 1H), 2.39 (t, *J* = 12.6 Hz, 1H), 2.10 (d, *J* = 2.8 Hz, 6H), 2.07 (d, *J* = 2.6 Hz, 3H), 2.00 (dd, *J* = 11.6, 3.8 Hz, 2H), 1.80 – 1.74 (m, 3H), 1.65 – 1.57 (m, 6H), 1.34 (d, *J* = 6.3 Hz, 3H), 1.25 (s, 7H), 1.22 (s, 10H), 1.20 (s, 1H), 1.14 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.92 (s, 3H), 0.86 (dd, *J* = 6.4, 2.2 Hz, 3H), 0.78 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.92, 176.80, 170.56, 170.30, 169.57, 165.68, 165.38, 165.14, 140.07, 133.42, 133.25, 133.01, 129.86, 129.79, 129.69, 129.37, 129.32, 129.26, 128.68, 128.58, 128.37, 128.22, 127.20, 122.15, 109.30, 105.71, 99.65, 97.46, 81.42, 80.82, 79.54, 77.26, 76.57, 76.00, 74.47, 72.80, 71.80, 70.38, 69.93, 66.87, 66.82, 63.15, 63.06, 62.14, 56.44, 50.05, 41.64, 40.29, 39.72, 38.85, 38.80, 38.72, 37.19, 36.86, 32.14, 31.91, 31.51, 31.43, 30.31, 29.88, 28.83, 27.20, 27.11, 26.93, 20.82, 20.76, 20.73, 20.70, 19.27, 17.48, 17.16, 16.30, 14.55. HRMS m/z calcd for $C_{81}H_{105}O_{24}$ $[M+H]^+$: 1461.6996, found: 1461.6924.

4.2.6 Diosgenyl α-L- rhamnopyranosyl-(1→2)-[(α-L-arabinofuranosyl)- (1→4)]-β-D-glucopyranoside (3)

Carried out using same procedure as for **1** to give **3** (polyphyllin D) (90% yield) as a white solid. $[\alpha]_D$ +116° (*c* 0.52, CH_3OH). ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$) δ 5.42 (d, *J* = 2.4 Hz, 1H), 5.28 (s, 1H), 5.09 (s, 1H), 4.54 (d, *J* = 7.6 Hz, 1H), 4.46 (q, 1H), 4.21-4.12 (m, 2H), 4.05 (s, 1H), 3.99 (s, 1H), 3.94 (t, *J* = 4.4 Hz, 1H), 3.91-3.87 (m, 1H), 3.84-3.65 (m, 6H), 3.59 (t, *J* = 9.6 Hz, 1H), 3.54-3.44 (m, 3H), 3.42-3.36 (m, 5H), 2.48 (d, *J* = 13.2 Hz, 1H), 2.34 (t, *J* = 11.6 Hz, 1H), 2.08-1.90 (m, 6H), 1.81 (t, *J* = 8.0 Hz, 3H), 1.73-1.55 (m, 9H), 1.42-1.28 (m, 12H), 1.09 (s, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.88-0.83 (m, 6H). ^{13}C NMR (101 MHz, $MeOD/CDCl_3$) δ δ 141.23, 122.22, 110.19, 109.36, 101.45, 99.98, 85.50, 82.37, 81.70, 78.75, 77.94, 77.46, 77.19, 75.88, 73.40, 71.86, 71.55, 69.14, 67.42, 62.98, 62.49, 61.37, 57.26, 51.03, 42.36, 40.93, 40.41, 39.03, 38.00, 37.52, 32.70, 32.27, 32.21, 31.91, 30.88, 30.26, 30.21, 29.33, 21.49, 19.54, 17.62, 17.24, 16.55, 14.63. HRMS m/z calcd for $C_{44}H_{70}O_{16}Na$ $[M+Na]^+$: 877.4562, found: 877.4566.

4.2.7 Ethyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside (15)

To a suspension of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-tri-chloroacetimidate **14** (1.04 g, 2.4 mmol), **13** (0.5 g, 2 mmol) and 4 Å MS (1.0 g) in dry CH_2Cl_2 (10 mL) at -78 °C, was slowly added TMSOTf (18 μL, 0.1 mmol). After being stirred for 2 h, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 8:1) gave **15** (0.97 g, 92%) as a white syrup. $[\alpha]_D$ -156° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) 5.44 (s, 1H), 5.26 (d, *J* = 1.2 Hz, 1H), 5.25-5.22 (m, 1H), 5.14 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.00 (t, *J* = 9.6 Hz, 1H), 4.15-4.05 (m, 2H), 4.05-3.98 (m, 1H), 3.81 (dq, *J* = 12.6, 6.2 Hz, 1H), 3.49 (dd, *J* = 9.8, 7.2 Hz, 1H), 2.63-2.56 (m, 1H), 2.52-2.45 (m, 1H), 2.09 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H), 1.46 (s,3H), 1.27-1.22 (m, 9H), 1,17-1.14 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.01, 169.94, 109.55, 95.85, 81.91, 79.39, 77.75, 76.86, 70.81, 69.71, 69.04, 66.81, 64.31, 27.87, 26.36, 24.35, 20.90, 20.76, 20.66, 18.08, 17.33, 14.54. HRMS m/z calcd for $C_{23}H_{36}O_{11}SNa$ $[M+Na]^+$: 543.1876, found: 543.1862.

4.2.8 Ethyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside (17)

To a solution of **15** (0.9 g, 1.7 mmol) in MeOH (15 mL) was added NaOMe (30 mg). After stirring for 2 h at room temperature, the reaction mixture was neutralized with Dowex 50W (H^+), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2:CH_3OH, 30:1) to give ethyl α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside as white solid. Then, to the solution of the ethyl α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside in 2,2-dimethoxypropane (10 mL), $TsOH{\cdot}H_2O$ (55 mg, 0.29 mmol) was added and stirred for 2 h at -10 °C, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. The residue was applied to silica gel chromatography (petroleum ether-EtOAc, 6:1) to afford **16** as a syrup (0.68 g, 90%).

To a suspension of 2,3,4-tri-O-acetyl -α-L-rhamnopyranosyl-tri-chloroacetimidate **14** (1.0 g, 2.3 mmol), **16** (0.50 g, 1.15 mmol) and 4 Å MS (1.0 g) in dry CH_2Cl_2 (8 mL) at -78 °C, was slowly added TMSOTf (10 μL, 0.06 mmol). After being stirred for 0.5 h, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 7:1) gave **17** (0.67 g, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (s, 1H), 5.53 (s, 1H), 5.35 (br s, 1H), 5.30 (s, 1H), 5.21 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.07 (t, *J* = 12.0 Hz, 1H), 4.22-4.13 (m, 4H), 4.06-3.99 (m, 1H), 3.88 (dq, *J* = 12.4, 6.0 Hz, 1H), 3.72 (dq, *J* = 12.4, 6.2 Hz, 1H), 3.63 (dd, *J* = 9.8, 7.6 Hz, 1H), 3.54 (dd, *J* = 9.6, 7.2 Hz, 1H), 2.67 (dq, *J* = 14.4, 7.2 Hz, 1H), 2.55 (dq, *J* = 14.8, 7.4 Hz, 1H), 2.16 (s, 3H), 2.06 (s, 3H), 1.98 (m, 3H), 1.54 (d, *J* = 9.4 Hz, 6H), 1.35 – 1.27 (m, 15H), 1.22 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.94, 169.91, 169.80, 130.77, 128.63, 109.36, 109.23, 95.72, 95.38, 79.22, 78.10, 77.77, 76.76, 76.23, 70.67, 69.53, 68.97, 66.62, 64.36, 64.06, 27.78, 27.68, 26.29, 26.14, 24.19, 20.75, 20.61, 20.53, 17.88, 17.63, 17.19, 14.43. HRMS m/z calcd for $C_{32}H_{50}O_{15}SNa$ $[M+Na]^+$: 729.2768, found: 729.2784.

4.2.9 Ethyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene (1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-1-thio-α-L-rhamnopyranoside (19)

To a solution of **17** (0.5 g, 0.7 mmol) in CH_3OH (20 mL) in was added NaOMe (30 mg). After stirring for 2 h at room temperature, the reaction mixture was neutralized with Dowex 50W (H^+), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether-EtOAc, 8:1) to give *Ethyl α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene (1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-1-thio-α-L-rhamnopyranoside* as white solid. Then, to the solution of the ethyl α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-1-thio-α-L-rhamnopyranoside in 2,2-dimethoxypropane (5 mL), $TsOH{\cdot}H_2O$ (30 mg, 0.16 mmol) was added and stirred for 2 h at -10 °C, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. The residue was applied to silica gel chromatography (petroleum ether-EtOAc, 6:1) to afford **18** as white foam (0.39 g, 88%).

To a suspension of 2,3,4-tri-O-acetyl -α-L- rhamnopyranosyl-tri-chloroacetimidate **14** (0.25 g, 0.58 mmol), **18** (0.30 g, 0.48 mmol) and 4 Å MS (1.0 g) in dry CH_2Cl_2 (8 mL) at -78 °C, was slowly added TMSOTf (18 μL, 0.1 mmol). After being stirred for 2 h, the reaction was quenched with Et_3N (0.1 mL) and then filtered and concentrated. The residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 8:1) gave **19** (0.36 g, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (s, 2H), 5.52 (s, 1H), 5.35 (s, 1H), 5.31 (s, 1H), 5.21 (dd, *J* = 10.2, 2.8 Hz, 1H), 5.07 (t, *J* = 10.0 Hz, 1H), 4.31 (t, *J* = 6.6 Hz, 1H), 4.24 – 4.10 (m, 6H), 4.01 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.87 (dt, *J* = 12.8, 6.4 Hz, 1H), 3.73-3.61 (m, 3H), 3.53 (dd, *J* = 9.2, 7.4 Hz, 1H), 2.66 (dt, *J* = 14.4, 7.2 Hz, 1H), 2.59 – 2.47 (m, 1H), 2.16 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.57 – 1.51 (m, 8H), 1.35 – 1.26 (m, 21H), 1.21 (d, *J* = 6.0 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.04, 170.01, 169.93, 132.28, 130.89, 128.81, 109.56, 109.37, 109.34, 95.94, 95.67, 95.56, 79.46, 78.43, 78.31, 77.99, 76.93, 76.88, 76.48, 76.43, 76.39, 70.86, 69.70, 69.14, 66.80, 64.59, 64.43, 64.19, 30.54, 27.97, 27.91, 26.48, 26.32, 24.39, 20.94, 20.80, 20.71, 18.00, 17.81, 17.36, 14.62. HRMS m/z calcd for $C_{41}H_{64}O_{19}SNa$ $[M+Na]^+$: 915.3660, found: 915.3646.

4.2.10 Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-[2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-O-pivaloyl-β-D-glucopyranoside (20)

To a suspension of accept **9** (209 mg, 174 μmol), the thioglycoside donor **15** (181 mg, 348 μmol), and 4 Å MS (250 mg) in dry CH_2Cl_2 (10 mL) at -10 °C, was added NIS (78 mg, 347 μmol) and AgOTf (13 mg, 51 μmol). After being stirred for 0.5 h at this temperature, the reaction was quenched with Et_3N (0.1 mL), and then filtered and concentrated. The products were isolated by silica gel column chromatography (3:1, petroleum ether–EtOAc) to give **20** (250 mg, 87%) as white foam. $[\alpha]_D$ -42.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.6 Hz, 2H), 7.85 (d, *J* = 7.6 Hz, 2H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.43 (dd, *J* = 13.8, 5.4 Hz, 3H), 7.32 (d, *J* = 7.3 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 2H), 7.16 (t, *J* = 7.6 Hz, 2H), 5.72 – 5.64 (m, 1H), 5.57 – 5.49 (m, 2H), 5.37 (d, *J* = 8.6 Hz, 2H), 5.21 (d, *J* = 10.7 Hz, 2H), 5.14 – 4.95 (m, 5H), 4.65 (dd, *J* = 14.4, 6.8 Hz, 2H), 4.40 – 4.33 (m, 1H), 4.29 – 4.17 (m, 3H), 4.11 – 4.06 (m, 1H), 3.99 (t, *J* = 6.6 Hz, 3H), 3.86 (d, *J* = 5.4 Hz, 1H), 3.80 (s, 2H), 3.76 – 3.64 (m, 3H),

3.58 (s, 1H), 3.39 (t, *J* = 8.6 Hz, 2H), 3.30 (d, *J* = 11.2 Hz, 1H), 2.49 (d, *J* = 11.0 Hz, 1H), 2.32 (t, *J* = 11.8 Hz, 1H), 2.07 (s, 3H), 1.97 (s, 6H), 1.87 (s, 3H), 1.54 (dt, *J* = 14.2, 7.0 Hz, 8H), 1.42 (s, 5H), 1.25 (s, 5H), 1.17 (d, *J* = 8.0 Hz, 17H), 1.09 (s, 9H), 0.90 – 0.85 (m, 9H), 0.72 (d, *J* = 7.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.03, 176.85, 171.31, 170.06, 170.01, 169.90, 167.77, 165.73, 165.33, 165.11, 140.10, 133.40, 133.27, 132.98, 132.37, 130.97, 129.91, 129.85, 129.75, 129.49, 129.37, 128.89, 128.60, 128.40, 128.23, 122.19, 109.69, 109.34, 99.57, 97.60, 96.21, 96.10, 80.86, 79.53, 77.69, 77.30, 76.27, 76.01, 75.79, 74.81, 72.29, 71.89, 70.92, 70.44, 69.92, 69.80, 69.11, 66.97, 66.91, 65.62, 64.41, 62.17, 56.48, 50.09, 41.67, 40.33, 38.93, 36.89, 32.19, 31.95, 31.54, 31.47, 30.69, 30.63, 30.36, 29.93, 29.75, 28.87, 27.84, 27.26, 27.07, 26.27, 21.07, 21.01, 20.89, 20.73, 19.30, 19.24, 19.18, 17.98, 17.53, 17.48, 17.21, 16.34, 14.60, 13.79, 13.76. HRMS m/z calcd for $C_{91}H_{120}O_{28}Na$ $[M+Na]^+$: 1683.7864, found: 1683.7833.

4.2.11 Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)[2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-O-pivaloyl-β-D-glucopyranoside (21)

To a suspension of accept **9** (80 mg, 67 μmol), the thioglycoside donor **17** (57 mg, 80 μmol), and 4 Å MS (100 mg) in dry CH_2Cl_2 (5 mL) at -10 °C, was added NIS (18 mg, 80 μmol) and AgOTf (7 mg, 27 μmol). After being stirred for 0.5 h at this temperature, the reaction was quenched with Et_3N (0.1 mL), and then filtered and concentrated. Chromatography (3:1, petroleum ether–EtOAc) to give **21** (63 mg, 51%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.4 Hz, 2H), 7.92 (d, *J* = 7.4 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.73 – 7.71 (m, 1H), 7.61 (t, *J* = 6.2 Hz, 1H), 7.53 – 7.50 (m, 2H), 7.40 (d, *J* = 7.0 Hz, 1H), 7.32 (t, *J* = 7.8 Hz, 2H), 7.24 (t, *J* = 7.6 Hz, 2H), 5.76 (dd, *J* = 10.1, 3.2 Hz, 1H), 5.63 (s, 1H), 5.61 (d, *J* = 3.8 Hz, 1H), 5.58 (d, *J* = 3.4 Hz, 1H), 5.45 – 5.37 (m, 3H), 5.34 (s, 2H), 5.31 (d, *J* = 2.1 Hz, 1H), 5.21 (dd, *J* = 10.1, 3.4 Hz, 2H), 5.17 (s, 1H), 5.13 (s, 1H), 5.07 (td, *J* = 9.6, 3.0 Hz, 2H), 4.75 – 4.68 (m, 2H), 4.31 (t, *J* = 6.6 Hz, 3H), 4.19 (dd, *J* = 11.4, 6.0 Hz, 2H), 4.15 – 4.05 (m, 4H), 3.98 – 3.94 (m, 1H), 3.86-3.69 (m, 5H), 3.52 (dd, *J* = 8.8, 6.4 Hz, 4H), 3.37 (d, *J* = 10.8 Hz, 1H), 2.55 (d, *J* = 13.2 Hz, 1H), 2.37 (d, *J* = 12.0 Hz, 1H), 2.16 (d, *J* = 1.4 Hz, 6H), 2.05 (s, 6H), 1.98 (s, 6H), 1.72 (d, *J* = 2.2 Hz, 3H), 1.51 (d, *J* = 6.6 Hz, 8H), 1.34 (s, 9H), 1.24 (t, *J* = 4.1 Hz, 14H), 1.17 (s, 9H), 0.99 – 0.92 (m, 12H), 0.79 (d, *J* = 8.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.04, 176.84, 170.01, 167.72, 165.67, 165.32, 165.08, 140.06, 133.39, 132.31, 130.92, 129.86, 129.78, 129.71, 129.38, 129.26, 128.84, 128.57, 128.35, 128.19, 122.12, 109.56, 109.35, 109.29, 95.94, 95.52, 80.81, 79.46, 77.93, 77.25, 76.40, 76.23, 75.90, 75.30, 74.31, 74.03, 72.48, 72.07, 71.81, 70.96, 70.36, 69.93, 69.73, 69.13, 66.81, 65.57, 64.28, 62.11, 56.43, 53.44, 50.03, 41.62, 40.27, 39.71, 38.85, 37.16, 36.84, 32.13, 31.49, 30.57, 30.30, 29.88, 29.70, 27.87, 27.83, 27.22, 27.18, 27.15, 27.02, 26.29, 26.24, 20.98, 20.83, 20.74, 19.25, 19.19, 17.84, 17.47, 17.39, 17.15, 16.29, 14.55, 13.74. HRMS m/z calcd for $C_{100}H_{134}O_{32}Na$ $[M+Na]^+$: 1869.8756, found: 1869.8723.

4.2.12 Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-O-isopropylidene-α-L-rhamnopyranosyl-(1→4)-[2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-O-pivaloyl-β-D-glucopyranoside (22)

To a suspension of accept **9** (100 mg, 83 μmol), the thioglycoside donor **17** (89 mg, 100 μmol), and 4 Å MS (100 mg) in dry CH_2Cl_2 (5 mL) at -10 °C, was added NIS (22 mg, 98 μmol) and AgOTf (9 mg, 33 μmol). After being stirred for 0.5 h at this temperature, the reaction was quenched with Et_3N (0.1 mL), and then filtered and concentrated. Chromatography (3:1, petroleum ether–EtOAc) afforded the compound **22** (93 mg, 60%) as white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.4 Hz, 2H), 7.92 (d, *J* = 7.4 Hz, 1H), 7.78 (d, *J* = 7.4 Hz, 2H), 7.71 (dt, *J* = 7.2, 3.6 Hz, 1H), 7.62 (dd, *J* = 13.8, 7.2 Hz, 1H), 7.54 – 7.47 (m, 4H), 7.40 (d, *J* = 7.0 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.24 (t, *J* = 7.4 Hz, 2H), 5.76 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.65 – 5.56 (m, 4H), 5.46 – 5.29 (m, 5H), 5.24 – 5.16 (m, 2H), 5.09 (dd, *J* = 19.6, 9.6 Hz, 2H), 4.77 – 4.67 (m, 1H), 4.47 – 4.35 (m, 2H), 4.30 (dd, *J* = 11.9, 5.2 Hz, 3H), 4.22 (dt, *J* = 10.5, 3.8 Hz, 2H), 4.17 – 4.08 (m, 4H), 4.00 – 3.95 (m, 1H), 3.92 – 3.46 (m, 12H), 3.39 (t, *J* = 10.8 Hz, 1H), 2.56 (d, *J* = 13.0 Hz, 1H), 2.39 (t, *J* = 11.6 Hz, 1H), 2.16 (s, 3H), 2.05 (s, 3H), 1.98 (s, 4H), 1.80 (d, *J* = 6.4 Hz, 2H), 1.72 (dt, *J* = 14.7, 6.8 Hz, 4H), 1.60 (s, 4H), 1.53 (s, 9H), 1.47 – 1.40 (m, 5H), 1.33 (d, *J* = 5.8 Hz, 8H), 1.30 – 1.19 (m, 30H), 1.17 (s, 6H), 0.99 – 0.94 (m, 8H), 0.79 (d, *J* = 6.8 Hz, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.06, 176.85, 170.05, 169.99, 167.72, 165.67, 165.35, 165.08, 140.06, 133.41, 132.98, 132.30, 130.92, 129.94, 129.86, 129.78, 129.70, 129.37, 129.29, 129.22, 128.84, 128.56, 128.46, 128.35, 128.20, 122.12, 109.88, 109.60, 109.38, 109.29, 99.33, 97.25, 96.44, 95.95, 95.56, 80.80, 79.44, 78.36, 78.12, 78.00, 77.38, 77.26, 77.06, 76.88, 76.75, 76.41, 76.25, 75.84, 75.45, 74.39, 72.63, 71.79, 70.91, 70.35, 69.95, 69.73, 69.17, 66.81, 65.56, 64.43, 64.17, 63.67, 62.11, 56.42, 50.03, 41.61, 40.27, 39.70, 38.85, 38.72, 37.16, 36.84, 32.12, 31.89, 31.48, 31.40, 30.56, 30.29, 30.19, 29.88, 29.69, 28.80, 28.59, 27.89, 27.85, 27.20, 27.02, 26.53, 26.35, 20.97, 20.83, 20.74, 19.25, 19.18, 17.83, 17.73, 17.47, 17.39, 17.15, 16.28, 14.55, 13.73. HRMS m/z calcd for $C_{109}H_{147}O_{36}$ $[M-H]^-$: 2031.9672, found: 2031.9677.

Typical procedure for deprotecting of compounds 20-22

A fully protected saponin **20-22** (100 mg) was dissolved in 80% HOAc and the solution was stirred at 80 ℃ for 5 h to remove isopropylidene protecting group. Then the solvent was evaporated to get a residue. The above residue was dissolved in THF-CH_3OH-H_2O (9 mL, v/v/v=1:1:1). NaOH (40 mg) was added and the mixture was stirred at 45 ℃ for 18 h to remove other protecting groups. The solution was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2:CH_3OH 10:1-3:1) to afford target compounds **4-6**.

4.2.13 Diosgenyl α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)] β-D-glucopyranoside (4)

Purification by silica gel column chromatography (CH_2Cl_2: CH_3OH, 4:1) gave a white solid (56 mg, 93%): R_f 0.38 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ 105° (*c* 0.5, MeOH) ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$) δ 5.37 (s, 1H), 5.18 (d, *J* = 7.5 Hz, 2H), 4.54 (s, 1H), 4.48 (d, *J* = 7.7 Hz, 1H), 4.39 (dd, *J* = 14.7, 7.2 Hz, 1H), 4.10 (dt, *J* = 12.2, 6.1 Hz, 1H), 4.01 (dt, *J* = 12.5, 6.3 Hz, 1H), 3.94 (s, 2H), 3.82 – 3.74 (m, 3H), 3.72 – 3.50 (m, 9H), 3.48 – 3.34 (m, 5H), 2.43 (d, *J* = 10.2 Hz, 1H), 2.28 (t, *J* = 11.7 Hz, 1H), 2.04 – 1.82 (m, 6H), 1.80 – 1.71 (m, 2H), 1.69 – 1.36 (m, 11H), 1.33 – 1.21 (m, 14H), 1.14 (ddd, *J* = 28.6, 17.2, 7.6 Hz, 3H), 1.03 (s, 3H), 0.99 – 0.84 (m, 6H), 0.79 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, MeOD/$CDCl_3$) δ

139.90, 120.84, 108.80, 101.17, 100.76, 100.35, 98.60, 80.34, 78.91, 77.86, 77.47, 76.05, 74.67, 72.03, 71.91, 70.96, 70.46, 70.17, 68.51, 67.85, 67.27, 66.04, 61.67, 60.04, 59.99, 55.91, 52.87, 49.71, 41.00, 39.56, 39.05, 37.62, 36.65, 36.16, 31.34, 30.87, 30.55, 29.53, 28.85, 27.97, 20.12, 18.12, 16.80, 16.24, 16.12, 15.81, 15.11, 13.19. HRMS m/z calcd for $C_{51}H_{82}O_{20}Na$ $[M+Na]^+$: 1037.5297, found: 1037.5290.

4.2.14 Diosgenyl α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)] β-D-glucopyranoside (5)

Purification by silica gel column chromatography (CH_2Cl_2: CH_3OH, 4:1) gave a white solid (56 mg, 90%): R_f 0.30 (4:1 CH_2Cl_2-CH_3OH); $[\alpha]_D$ +103° (*c* 0.52, CH_3OH); ^1H NMR (400 MHz, d-DMSO) δ 5.31 (s, 1H), 5.05 (s, 2H), 5.02 (d, *J* = 4.8 Hz, 1H), 5.00 (s, 1H), 4.92 – 4.86 (m, 2H), 4.83 – 4.55 (m, 11H), 4.37 (d, *J* = 7.3 Hz, 1H), 4.26 (d, *J* = 7.2 Hz, 1H), 4.02 – 3.88 (m, 1H), 3.70 (s, 3H), 3.64 – 3.47 (m, 8H), 3.18 (dd, *J* = 12.0, 6.7 Hz, 5H), 2.39 (d, *J* = 8.6 Hz, 1H), 2.13 (dd, *J* = 24.2, 11.7 Hz, 1H), 1.90 (s, 2H), 1.82 – 1.73 (m, 3H), 1.66 (s, 2H), 1.50 (dt, *J* = 38.0, 12.4 Hz, 8H), 1.31 – 1.20 (m, 5H), 1.11 (dt, *J* = 17.3, 8.7 Hz, 18H), 0.90 (dd, *J* = 21.1, 14.4 Hz, 9H), 0.72 (d, *J* = 4.0 Hz, 6H). ^{13}C NMR (101 MHz, d-DMSO) δ 140.41, 121.42, 108.55, 101.15, 100.45, 100.15, 98.34, 93.88, 80.32, 79.02, 78.66, 78.07, 77.98, 77.84, 76.67, 76.36, 76.12, 76.00, 75.45, 72.08, 71.60, 71.21, 70.87, 70.71, 70.47, 69.03, 68.04, 67.27, 66.75, 66.03, 61.90, 55.87, 49.68, 41.21, 40.23, 40.02, 39.81, 39.60, 39.39, 39.18, 38.97, 37.70, 36.93, 36.52, 31.64, 31.10, 29.94, 29.13, 28.60, 20.50, 19.10, 18.55, 18.28, 17.93, 17.22, 16.15, 14.80. HRMS m/z calcd for $C_{57}H_{92}O_{24}Na$ $[M+Na]^+$: 1183.5876, found: 1183.5823.

4.2.15 Diosgenyl α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (6)

Purification by silica gel column chromatography (DCM: MeOH, 4:1) gave a white solid (57 mg, 88%): R_f 0.26 (3:1 CH_2Cl_2-CH_3OH); $[\alpha]_D$ +101° (*c* 0.16, CH_3OH) ^1H NMR (400 MHz, $CD_3OD/CDCl_3$) δ 5.33 (s, 1H), 5.13 (s, 2H), 5.09 (s, 1H, H-1$^{r'''' (g')}$), 5.08 (s, 1H, H-1 $^{r'''' (g')}$), 4.44 (d, *J* = 7.8 Hz, 1H), 4.36 (dd, *J* = 15.0, 7.2 Hz, 1H), 4.11 – 4.04 (m, 1H), 3.99 (dd, *J* = 9.2, 6.2 Hz, 1H), 3.92 (d, *J* = 8.6 Hz, 2H), 3.87 (s, 2H), 3.76 (d, *J* = 10.4 Hz, 2H), 3.73 – 3.65 (m, 6H), 3.61 (dd, *J* = 10.0, 2.4 Hz, 2H), 3.56 – 3.46 (m, 6H), 3.40 – 3.33 (m, 3H), 3.32 (s, 1H), 3.28 (dd, *J* = 3.0, 1.6 Hz, 5H), 2.39 (d, *J* = 10.4 Hz, 1H), 2.25 (t, *J* = 12.4 Hz, 1H), 1.95 (dd, *J* = 11.0, 6.4 Hz, 2H), 1.89 – 1.80 (m, 3H), 1.71 (dd, *J* = 9.2, 5.8 Hz, 2H), 1.64 – 1.46 (m, 9H), 1.44 – 1.36 (m, 2H), 1.33 – 1.17 (m, 25H), 1.16 – 1.09 (m, 2H), 1.04 – 0.98 (m, 4H), 0.92 (q, *J* = 9.6 Hz, 5H), 0.83 (dd, *J* = 11.2, 4.4 Hz, 2H), 0.75 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$) δ 170.12, 141.35, 122.28, 110.23, 102.72, 102.64, 102.61, 102.14, 101.82, 100.08, 81.77, 81.00, 80.54, 79.22, 79.01, 78.87, 78.69, 78.36, 77.50, 76.13, 73.48, 73.40, 72.58, 72.48, 72.28, 71.85, 71.61, 69.94, 69.32, 68.69, 68.46, 67.49, 63.12, 61.48, 57.35, 51.15, 49.65, 49.44, 49.23, 49.01, 48.80, 48.59, 48.37, 48.16, 42.45, 41.01, 40.49, 39.07, 38.09, 37.61, 32.78, 32.63, 32.31, 32.00, 30.97, 30.30, 29.42, 23.32, 21.57, 19.57, 18.26, 17.70, 17.57, 17.25, 16.56, 14.64. HRMS m/z calcd for $C_{63}H_{102}O_{28}Na$ $[M+Na]^+$: 1329.6450, found: 1329.6279.

4.3 Cytotoxicity test

The human hepatocellular carcinoma cell lines (HepG2) and human normal liver cells L02 were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) and were maintained in Dulbecco's modified Eagle's medium. A total of 8000 cells in the logarithmic phase/well were seeded on 96-well plates with supplemented culture medium (100 μL/well) and incubated at 37 ℃ in a humidified atmosphere containing 5% CO_2 for 24 h. Cultured medium was removed and replaced with serum free supplemented medium (100 μL/well). After the cells were incubated with different concentrations of saponins 1-7 for 24 h, MTT (0.5 mg/mL) was diluted in phosphate-buffered saline (PBS) and added to each well with a further incubation for 4 h. All cytotoxicity tests were run parallel with a set of negative controls: cells without addition of complex for comparison. Finally, the cells were dissolved with 100 μL of dimethyl sulfoxide (DMSO) and then analyzed in a multiwall plate reader at 570 nm (BioTek Instruments, Inc.).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/XXXXX

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