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Synthesis, cytotoxicity, and hemolytic activity of 6′-O-substituted dioscin derivatives

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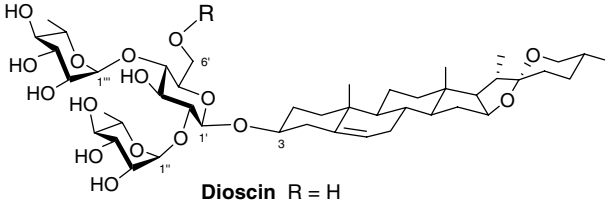
Abstract—Dioscin derivatives (**1**–**12**) with a variety of substitutions at the 6′-OH of the chacotriosyl residue and the 3′,6′-anhydrosaponin derivatives (**26**, **30**, and **32**) were synthesized. All these derivatives showed much lower cytotoxicity than that of the parent dioscin, while their hemolytic activities were partially retained depending on the various 6′-O-substitutions.

Keywords: Steroid saponins; Synthesis; Hemolytic activity; Cytotoxicity; Dioscin

1. Introduction

Two quite common features of the spirostan saponins, which occur widely and abundantly in plants, are their hemolytic activity toward erythrocytes and their inhibitory activity against the growth of tumor cells.[1] The potency of these two activities is highly dependent on the sugar residues of the saponins.[2,3] Dioscin, diosgenin-3-yl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (chacotrioside), which represents one of the most common plant spirostan saponins, is among the most potent ones in both hemolytic and cytotoxic activities.[2,3] In addition, facile synthetic approaches toward dioscin have been well developed.[4] Therefore, we have been trying to employ dioscin as a lead structure to decipher the structure–activity relationships and mechanism of action of spirostan saponins.[3,5] To this end, all the eight possible monomethylated derivatives of dioscin were synthesized. It was found that only the 6′-*O*-methyl derivative (**1**) and the 4‴-*O*-methyl derivative could retain the cytotoxicity of dioscin partially, but other mono-*O*-methyl isomers were nearly inactive.[5a] Further studies confirmed that substitution on the 4‴-OH of dioscin with a variety of groups hardly altered their hemolytic and cytotoxic potencies.[5d] Attempts to prepare the corresponding 6′-O-substituted-dioscin derivatives have been problematic. Alternatively, the 6′-*N*-acyl-6′-deoxy-dioscin derivatives were readily prepared, but these derivatives were found largely inactive.[5d] Herein we report how we solved the problems in the synthesis



Dioscin R = H

1 R = Me
2 R = Et
3 R = All
4 R = Bn
5 R = $CH_2CH_2N_3$
6 R = $CH_2CH_2NH_2$
7 R = $CH_2CH_2NHCOCH_3$
8 R = $CH_2CH_2NHCOCF_3$
9 R = $CH_2CH_2NHCOPh$
10 R = $CH_2CH_2NHCO(CH{=}CH)_2CH_3$
11 R = $CH_2CH_2NHCO(CH_2)_{12}CH_3$
12 R = $CH_2CH_2NHDansyl$

Figure 1. Dioscin and its 6′-O-substituted derivatives **1**–**12**.

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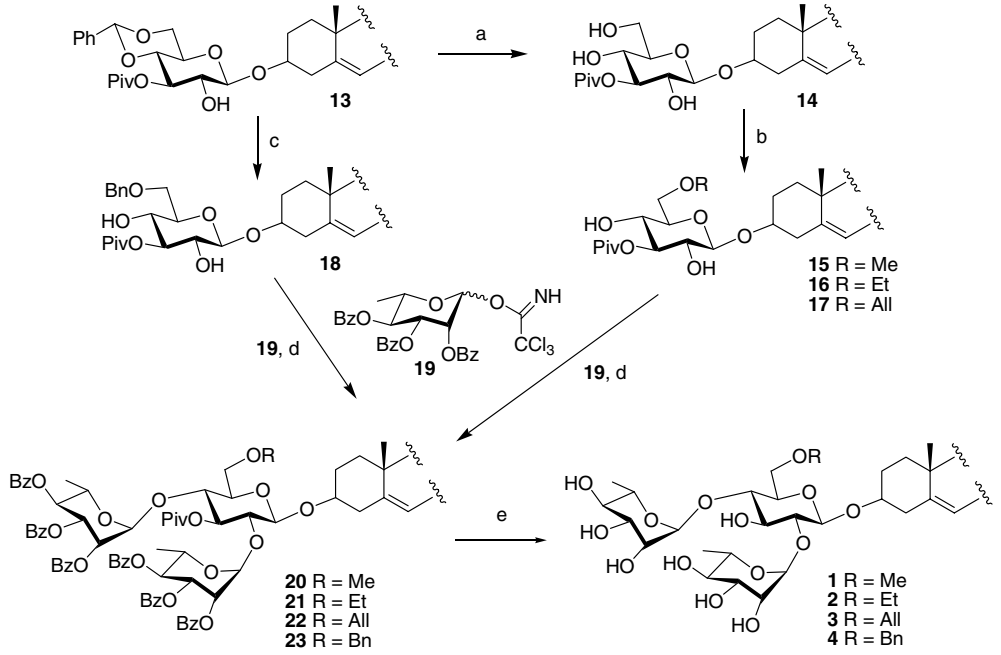
of the desired 6′-O-substituted-dioscin derivatives (e.g., **1**–**12**, Fig. 1) and the hemolytic and cytotoxic activities of these compounds.

2. Results and discussion

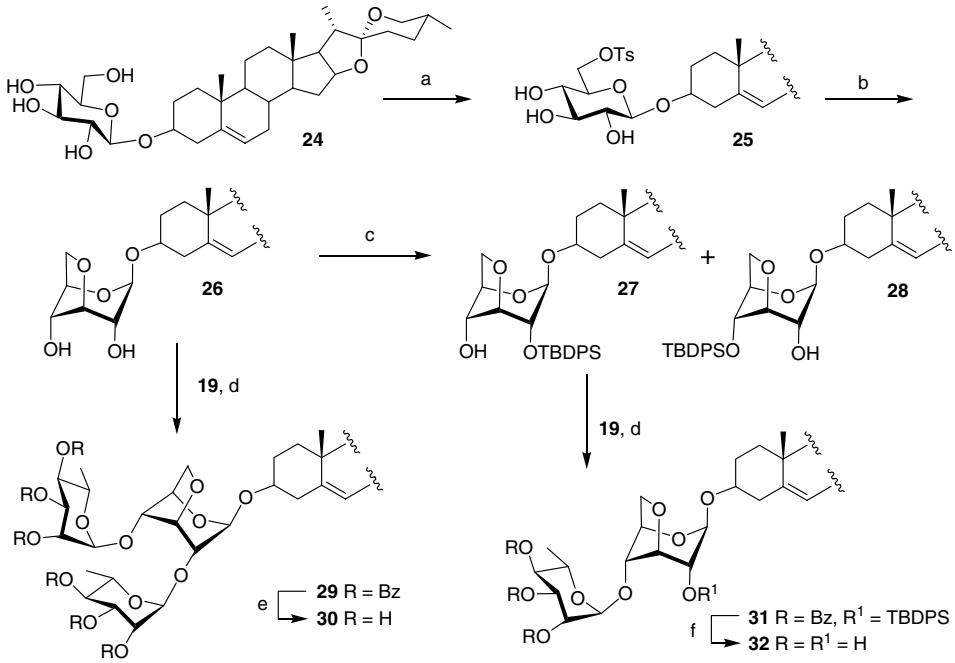
The previous approach to the synthesis of the 6′-*O*-methyl-dioscin (**1**) employed a tin-mediated selective methylation of the primary 6′-OH on the triol derivative **14** (Scheme 1). The yield for this step of conversion (**14**→**15**) was very low (29%).[5a] After modification of the reaction conditions, the methylation yield was improved to 52%. However, substitution with iodoethane under similar conditions provided the corresponding 6′-*O*-ethyl derivative **16** in a very low 8% yield. Allylation (with the more electrophilic allyl bromide) gave the 6′-*O*-allyl product **17** in a better 37% yield. The 6′-*O*-benzyl derivative **18** was prepared, also in low yield (19%), via a reductive opening of the 4′,6′-*O*-benzylidene on **13**; however, no attempt was made to improve this transformation. Glycosylation of the resulting 2′,4′-diols (**15**–**18**) with 2,3,4-tri-*O*-benzoyl-L-rhamnopyranosyl trichloroacetimidate (**19**)[5] under the promotion of TMSOTf provided the corresponding trisaccharides **20**–**23** in yields of 80–94%. Final removal of the benzoyl and pivaloyl groups with LiOH furnished the desired 6′-*O*-alkyl dioscin derivatives **1**–**4** in good yield.

Given the difficulty in the selective alkylation of the 6′-OH of the saponin derivatives, we attempted to synthesize the desired 6′-O-substituted-dioscin derivatives via a S_N2 substitution of a 6′-*O*-tosylate derivative (Scheme 2). Thus, diosgenin-3-yl β-D-glucopyranoside (**24**, trillin)[6] was subjected to selective sulfonylation at the primary 6′-OH with *p*-toluenesulfonyl chloride in pyridine; the desired 6′-*O*-tosyl derivative **25** was obtained in a satisfactory 69% yield. Although substitution of the 6′-*O*-tosylate with NaN_3 successfully led to the corresponding 6′-azide derivative in excellent yield,[5d] treatment of **25** with methanol (and other alcohols as well) under basic conditions provided 3′,6′-anhydride **26** as the predominant product.[7] Acyl protection of the hydroxyl groups on **25** could not survive in the subsequent substitution reaction, leading again to the 3′,6′-anhydro-derivatives. Attempts to protect the 2′,3′,4′-OHs on **25** with benzyl or silyl groups under mild basic conditions also led to the 3′,6′-anhydride formation.

Glycosylation of the resulting 2′,4′-diol **26** with rhamnopyranosyl trichloroacetimidate **19** under the promotion of TMSOTf provided the expected trisaccharide **29** in a moderate 33% yield. This result reflected the steric hindrance of the two axial hydroxyl groups in **26**. Treatment of **26** with 1.5 equiv of TBDPSCl in the presence of imidazole provided the monosilylated products **27** and **28** in equal amounts. Glycosidic coupling of **27** with rhamnopyranosyl trichloroacetimidate **19** led to disaccharide **31** in 65% yield. In comparison, glycosylation of **28** with **19** did not give any of the coupling product, implying the 2′-OH in saponin derivative **28** is more difficult to access than the 4′-OH in **27**. Removal



Scheme 1. Synthesis of the 6′-*O*-alkyl-dioscin derivatives **1**–**4**. Reagents and conditions: (a) *p*-TsOH·H_2O, MeOH, 40 °C, >76%; (b) $(Bn_3Sn)_2O$, MeI (EtI, or AllBr), TBAI, DMF, 52% (for **15**), 8% (for **16**), 37% (for **17**); (c) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, ~19%; (d) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C→rt, 80–94%; (e) LiOH·H_2O, 1:3 THF–MeOH, 55–83%.

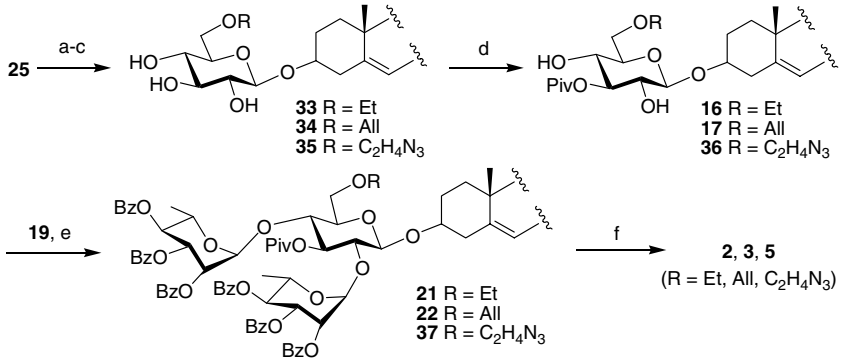


Scheme 2. Synthesis of the 3′,6′-anhydrodioscin derivative (**30**). Reagents and conditions: (a) TsCl, pyridine, 0 °C→rt, 69%; (b) NaOH, MeOH, 60 °C, 92%; (c) TBDPSCl, imidazole, CH_2Cl_2, 45% (for **27**), 45% (for **28**); (d) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C→rt, 33% (for **29**); 65% (for **31**); (e) NaOMe, MeOH–CH_2Cl_2, 43%; (f) TBAF, THF, then NaOMe, MeOH, rt, 94%.

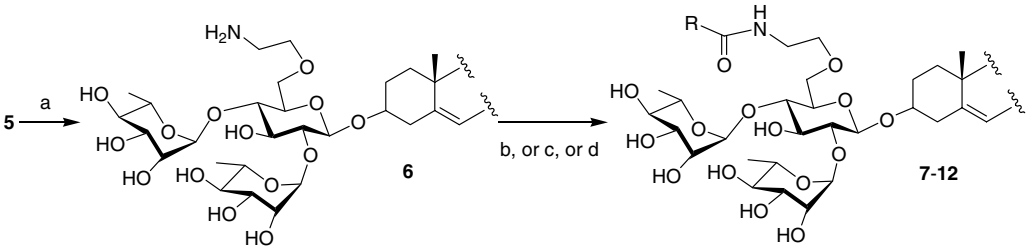
of the silyl and acyl protecting groups on **29** and **31** furnished the novel saponin derivatives **30** and **32** bearing a 3′,6′-anhydroglucose residue.

To prevent the 3′,6′-anhydro formation in the substitution reaction of the 6′-*O*-tosyl-glucopyranoside derivatives (i.e., **25**), acidic conditions shall be firstly applied to block the 2,3,4-OHs with a protecting group, that is, inert toward the subsequent basic conditions. Thus, triol **25** was treated with ethyl vinyl ether in the presence of pyridinium *p*-toluenesulfonate (PPTS) to afford a complex mixture of the corresponding 2,3,4-tri-*O*-EE (ethoxyethyl) derivatives, where the complexity is raised by the nascent chiral center in the ethoxyethyl group (Scheme 3). Subjection of the resulting mixture to alcohols (e.g., EtOH, AllOH, and 2-azidoethanol) in the presence of sodium in THF,[8] followed by removal of the ethoxyethyl group with 50% AcOH, provided the desired 6′-*O*-alkylated products (**33**–**35**) in good yields (56–71%) over three steps. Triols **33**–**35** were then subjected to selective protection with a pivaloyl group at the 3′-OH, giving 2′,4′-diol derivatives **16**, **17**, and **36** in good yields (73–82%). Finally, glycosylation of the diols with rhamnopyranosyl trichloroacetimidate **19**, followed by removal of the acyl protection, furnished, as expected, the target 6′-O-substituted dioscins **2**, **3**, and **5**.

The 6′-*O*-(2-azidoethyl)dioscin derivative **5** was expected to serve as a precursor to the facile access to a variety of the congeners via a selective acylation of the releasing amino group (Scheme 4).[5d] Thus, reduction of the azido group (in **5**) to the amino group with PPh_3



Scheme 3. Improved synthesis of the 6′-*O*-alkyldioscin derivatives. Reagents and conditions: (a) ethyl vinyl ether, PPTS, CH_2Cl_2, rt; (b) Na, EtOH (or AllOH, or 2-azidoethanol), THF, reflux; (c) 50% aq AcOH, 67% (for **33**, three steps), 71% (for **34**, three steps), 56% (for **35**, three steps); (d) PivCl, 1:1 pyridine–CH_2Cl_2, −10 °C→− 4 °C, 73–82%; (e) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C→rt, 84–97%; (f) LiOH·H_2O, 1:3 THF–MeOH, 40 °C, 79–83%.



Scheme 4. Synthesis of the 6′-*O*-(2-acyl-*N*-ethyl)dioscin derivatives (**6**–**12**). Reagents and conditions: (a) PPh_3, 4:1 THF–H_2O, 60 °C, 94%; (b) AcCl (or $(CF_3CO)_2O$, or BzCl), Et_3N, MeOH, 82–96% (for two steps); (c) dansyl chloride, $NaHCO_3$, MeOH, 77% (for two steps); (d) tetradecanoic acid (or sorbic acid), $(COCl)_2$, DMF, CH_2Cl_2; then Et_3N, MeOH, 87–93% (for two steps).

provided 6′-*O*-(2-aminoethyl)dioscin **6** in an excellent 94% yield. Otherwise, direct treatment of crude **6** with a variety of acyl chlorides (i.e., acetyl, benzoyl, dansyl, tetradecanoyl, or (2*E*,4*E*)-hexa-2,4-dienoyl chloride) or anhydride [$(CF_3CO)_2O$] in the presence of Et_3N (or $NaHCO_3$) in methanol furnished the desired 6′-*O*-(2-acylaminoethyl)dioscin derivatives **7**–**12** in excellent yields (77–96%).

The inhibitory activity of the synthesized 6′-O-substituted dioscin derivatives (**1**–**12**) and the 3′,6′-anhydrosaponins (**26**, **30**, and **32**) against the growth of three tumor cell lines, that is, HGC-27 (human gastric carcinoma cell), A549 (human lung carcinoma cell), and BGC-823 (human gastric cancer cell) were evaluated following a standard MTT assay with dioscin as a positive control.[9] The hemolytic activity of these saponin derivatives, expressed as the concentrations that cause 50% hemolysis of human erythrocytes (HD_{50}), were measured according to a slightly modified literature protocol as described before.[3,11] The results are listed in Table 1.

Table 1. Hemolytic and cytotoxic activities of dioscin derivatives **1**–**12**, **26**, **30**, and **32**[a]

Compound	Hemolytic activity HD_{50} (μM)	Inhibition rate at 10 μM		
		HGC-27	A549	BGC-823
1	≫50	21%	10%	16%
2	44.4 ± 1.1	25%	13%	11%
3	55.1 ± 1.0	ND	20%	32%
4	>50	17%	12%	15%
5	≫100	50%	21%	37%
6	32.9 ± 1.0	18%	29%	23%
7	16.6 ± 0.6	16%	15%	NI
8	8.8 ± 0.1	17%	14%	13%
9	9.3 ± 0.6	ND	ND	ND
10	14.1 ± 0.2	22%	15%	16%
11	≫50	11%	16%	14%
12	7.3 ± 0.8	15%	13%	14%
26	≫100	33%	37%	31%
30	ND	NI	NI	NI
32	56.5 ± 0.7	NI	NI	NI
Dioscin	3.2 ± 0.2	76% at 5 μM	88% at 5 μM	67% at 5 μM

[a] ND = Not detected; precipitation of the compound was found during measurement. NI = No inhibition was detected.

None of the dioscin derivatives with a 6′-O-substitution (**1**–**12**, **26**, **30**, and **32**) showed appreciable inhibition activity at a concentration of 10 μM toward the three tumor cell lines, while the parent dioscin showed significant activity at 5 μM. Precipitation was found for most of these compounds at higher concentrations (>25 μM) during cell tests, therefore, the IC_{50} values could not be determined. The 6′-O-substitution also significantly lowered the hemolytic activity of dioscin; however, this influence is highly dependent on the substituted groups. It is noteworthy that compounds (e.g., **8**, **9**, and **12**) could retain a large part of the hemolytic activities of dioscin, but their cytotoxicities are only marginal. These results prove again that the hemolytic activity and cytotoxicity of saponins are not correlated.[3]

In summary, we have developed an effective approach to the synthesis of the 6′-O-alkylated dioscin derivatives in which the ready formation of the corresponding 3′,6′-anhydride is avoided. Interestingly, the 6′-O-monosubstitution significantly lowers the hemolytic and cytotoxic activities of the parent saponin.

3. Experimental

3.1. General methods

General methods for synthesis: see Ref. 10.

General methods for MTT assay of the cytotoxicity: see Ref. 9.

General methods for assay of the hemolytic activity: see Ref. 3.

3.2. Preparation of diosgenyl-*O*-methyl 2,4-di-*O*-α-L-rhamnopyranosyl-6-*O*-methyl-β-D-glucopyranoside (1)

3.2.1. Preparation of diosgenyl 6-*O*-methyl-3-*O*-pivaloyl-β-D-glucopyranoside (15). A mixture of triol **14** (132 mg, 0.20 mmol), $(Bn_3Sn)_2O$ (0.18 mL, 0.35 mmol), and 4 Å MS in anhyd DMF (2.5 mL) was stirred under N_2 for 20 min at rt and then warmed up to 80 °C for 5 h. After cooling down to room temperature, MeI (2.0 mL, 32 mmol) and TBAI (148 mg, 0.40 mmol) were added, and the stirring was continued for another 10 h at

30 °C. The mixture was then filtered and concentrated. Water and CH_2Cl_2 were added. The organic layer was washed with satd aq NaCl, dried with Na_2SO_4, and concentrated. The residue was subjected to silica gel column chromatography to provide **15** (70 mg, 52%) as a white solid.[5a]

3.2.2. Preparation of diosgenyl 6-*O*-methyl-3-*O*-pivaloyl-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (20). A mixture of diol **15** (265 mg, 0.39 mmol), imidate 19 (1.46 g, 2.36 mmol), and 4 Å MS (0.3 g) in anhyd CH_2Cl_2 (10 mL) under N_2 was cooled to 0 °C and stirred for 0.5 h. TMSOTf (7.7 μL, 0.040 mmol) was then added. The resulting mixture was stirred for an additional 0.5 h and then warmed up to room temperature for another 0.5 h. Et_3N was then added to quench the reaction. Filtration and concentration led to a residue that was applied to silica gel column chromatography (15:1 petroleum ether–EtOAc) to provide **20** (583 g, 94%) as a white solid.[5a]

3.2.3. Preparation of diosgenyl 6-*O*-methyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (1). A solution of **20** (174 mg, 0.11 mmol) and $LiOH{\cdot}H_2O$ (277 mg, 6.60 mol) in THF (2 mL) and MeOH (6 mL) was stirred at 40 °C overnight. The solvent was removed and H_2O (20 mL) was added to give a white solid that was filtered and purified by silica gel column chromatography (12:1 CH_2Cl_2–MeOH) to afford **1** (53 mg, 55%) as a white solid: $[\alpha]_D^{23}$ −94.8 (*c* 0.59, MeOH).[5a]

3.3. Diosgenyl 6-*O*-ethyl-3-*O*-pivaloyl-β-D-glucopyranoside (16)

A similar procedure used for the preparation of **15** was employed. Thus, treatment of **14** (660 mg, 1.00 mmol) with $(Bn_3Sn)_2O$ (1.0 mL, 2.0 mmol), followed by EtI (0.80 mL, 10 mmol) and TBAI (738 mg, 1.87 mmol), provided **16** (54 mg, 8%) as a white solid: $[\alpha]_D^{27}$ −74.6 (*c* 0.52, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 5.30 (br s, 1H, H-6), 4.84 (t, $J = 9.0$ Hz, 1H, H-3′), 4.40–4.33 (m, 2H, H-16, H-1′), 3.65–3.64 (m, 2H), 3.58–3.40 (m, 7H), 3.38–3.30 (m, 1H), 2.38–2.17 (m, 2H), 1.18 (s, 9H, Piv), 1.13 (t, $J = 6.9$ Hz, 3H, Et–CH_3), 0.95 (s, 3H, H-19), 0.90 (d, $J = 6.9$ Hz, 3H, H-21), 0.73 (m, 6H, H-18, H-27); HRESIMS: calcd for $C_{40}H_{65}O_9$ ($M+H^+$), 689.4629. Found: 689.4606.

3.4. Diosgenyl 6-*O*-allyl-3-*O*-pivaloyl-β-D-glucopyranoside (17)

A similar procedure used for the preparation of **15** was employed. Thus, treatment of **14** (330 mg, 0.5 mmol) with $(Bn_3Sn)_2O$ (0.5 mL, 1 mmol), followed by allyl bromide (2.6 mL, 3.0 mmol) and TBAI (369 mg, 1.00 mmol), provided **17** (131 mg, 37%) as a white solid: $[\alpha]_D^{27}$ −73.0 (*c* 0.50, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 5.87–5.76 (m, 1H, All–CH), 5.29 (br s, 1H, H-6), 5.23–5.11 (m, 2H, All–C=CH_2), 4.82 (t, $J = 9.3$ Hz, 1H, H-3′), 4.39–4.30 (m, 2H, H-16, H-1′), 3.98 (d, $J = 5.7$ Hz, 2H, All–CH_2), 3.69–3.66 (m, 2H), 3.65–3.38 (m, 5H), 3.34–3.27 (m, 1H), 2.34–2.13 (m, 2H), 1.18 (s, 9H, Piv), 0.95 (s, 3H, H-19), 0.90 (d, $J = 6.9$ Hz, 3H, H-21), 0.73–0.72 (m, 6H, H-18, H-27); HRESIMS: calcd for $C_{41}H_{65}O_9$ ($M+H^+$), 701.4629. Found: 701.4653.

3.5. Diosgenyl 6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (25)

To a stirred solution of trillin[6] (1.48 g, 2.57 mmol) in anhyd pyridine (15 mL) at 0 °C was added *p*-toluenesulfonyl chloride (1.48 g, 7.77 mmol) under N_2. The mixture was gradually warmed up to room temperature and stirred overnight. The resulting solution was poured into water (600 mL). The white precipitate was filtered, dried, and then applied to silica gel column chromatography (30:1 CH_2Cl_2–MeOH), affording **25** (1.30 g, 69%) as a while solid: $[\alpha]_D^{25}$ −94.4 (*c* 0.53, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.31 (dd, $J = 8.4$ Hz, 2H, Ar–H), 5.32 (br s, 1H, H-6), 4.45–4.20 (m, 4H), 3.51–3.25 (m, 16H), 2.42 (s, 3H, Ts–CH_3), 2.36–2.17 (m, 2H), 0.99–0.97 (m, 6H, H-19, H-21), 0.79 (br s, 6H, H-18, H-27); HRESIMS: calcd for $C_{40}H_{59}O_{10}S$ ($M+H^+$), 731.3829. Found: 731.3742.

3.6. Diosgenyl 3,6-anhydro-β-D-glucopyranoside (26)

Compound **25** (584 mg, 0.80 mmol) was added to a stirred solution of sodium (276 mg, 12 mmol) in MeOH (15 mL). After stirring at 60 °C for 1 h, the mixture was concentrated. The residue was applied to silica gel column chromatography, providing **26** (409 mg, 92%) as a white solid: $[\alpha]_D^{23}$ −159.6 (*c* 0.91, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.35 (br s, 1H, H-6), 5.25 (s, 1H, H-1′), 4.86 (t, $J = 5.7$ Hz, 1H, H-3′), 4.45–4.38 (m, 2H), 4.25–4.19 (m, 2H), 3.83 (d, $J = 6.0$ Hz, 2H), 3.57–3.45 (m, 2H), 3.40–3.33 (m, 1H), 2.42–2.36 (m, 1H), 2.30–2.21 (m, 1H), 1.01 (s, 3H, H-19), 0.97 (d, $J = 6.9$ Hz, 3H, H-21), 0.80–0.78 (m, 6H, H-18, H-27); ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.0, 122.1, 109.4, 109.2, 87.8, 83.9, 80.8, 80.7, 78.7, 73.7, 71.4, 66.8, 62.0, 56.4, 50.0, 41.6, 40.2, 39.7, 38.7, 37.1, 36.9, 32.1, 31.8, 31.4, 31.3, 30.2, 29.2, 28.7, 20.8, 19.3, 17.1, 16.3, 14.5; HRESIMS: calcd for $C_{33}H_{51}O_7$ ($M+H^+$), 559.3635. Found: 559.3673.

3.7. Diosgenyl 3,6-anhydro-2-*O*-(*tert*-butyldiphenylsilyl)-β-D-glucopyranoside (27)

To a solution of diol **26** (227 mg, 0.41 mmol) and imidazole (55 mg, 0.81 mmol) in anhyd CH_2Cl_2 (4 mL)

was added *tert*-butylchlorodiphenylsilane (0.16 mL, 0.61 mmol) in anhyd CH_2Cl_2 (1 mL) dropwise under N_2. After stirring at rt for 4 h, the mixture was concentrated. The residue was subjected to silica gel column chromatography (15:1 petroleum ether–EtOAc), affording **27** (147 mg, 45%) as a white solid: $[\alpha]_D^{23}$ −157.5 (*c* 0.69, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, $J = 6.6$ Hz, 2H, Ar–H), 7.68 (d, $J = 6.0$ Hz, 2H, Ar–H), 7.48–7.40 (m, 6H, Ar–H), 5.38 (br s, 1H, H-6), 5.11 (s, 1H, H-1′), 4.50–4.38 (m, 2H), 4.30 (d, $J = 9.6$ Hz, 1H), 4.23–4.20 (m, 1H), 4.15 (br s, 1H), 3.83–3.79 (m, 1H), 3.73 (br s, 1H), 3.67–3.47 (m, 3H), 3.42–3.35 (m, 1H), 2.48–2.43 (m, 1H), 2.27–2.19 (m, 1H), 1.10 (s, 9H, TBDPS-$(CH_3)_3$), 1.02 (s, 3H, H-19), 0.97 (d, $J = 6.9$ Hz, 3H, H-21), 0.80 (m, 6H, H-18, H-27); ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.7, 135.8 (2C), 135.5 (2C), 132.0, 131.6, 130.5, 130.4, 128.1 (2C), 128.0 (2C), 121.5, 109.3, 101.3, 80.8, 76.6, 73.4, 73.1, 72.6, 71.5, 70.6, 66.9, 62.2, 56.5, 50.2, 41.6, 40.3, 39.8, 38.3, 37.4, 37.0, 32.1, 31.9, 31.5, 31.4, 30.3, 29.5, 28.8, 26.8 (3C), 20.9, 19.4, 19.1, 17.1, 16.3, 14.5; MALDITOF MS: calcd for $C_{49}H_{68}O_7SiNa$ ($M+Na^+$), 819.4626. Found: 819.4637.

3.8. Diosgenyl 3,6-anhydro-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (29)

To a mixture of 2,3,4-tri-*O*-benzoyl-L-rhamnopyranosyl trichloroacetimidate **19** (372 mg, 0.60 mmol), diol **26** (112 mg, 0.20 mmol) and 4 Å MS in anhyd CH_2Cl_2 (5 mL) at 0 °C under N_2 was added TMSOTf (3.5 μL, 0.020 mmol). The mixture was allowed to warm up to room temperature and was stirred for another 0.5 h. Et_3N was added to quench the reaction. The resulting mixture was filtered and concentrated. The residue was applied to silica gel column chromatography (15:1 petroleum ether–EtOAc, 25:1 toluene–EtOAc) to provide **29** (97 mg, 33%) as a white solid: $[\alpha]_D^{23}$ +58.7 (*c* 1.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15–8.10 (m, 4H, Ar–H), 7.94–7.88 (m, 4H, Ar–H), 7.62–6.82 (m, 22H, Ar–H), 6.04 (br s, 1H, Rha–H-1), 5.92–5.75 (m, 5H), 5.51–5.47 (m, 2H), 5.32 (s, 1H, H-6), 5.28 (s, 1H, H-1′), 4.61–4.52 (m, 3H), 4.45–4.38 (m, 3H), 4.29 (d, $J = 9.9$Hz, 1H), 4.04 (br s, 1H), 3.99–3.79 (m, 1H), 3.85–3.74 (m, 1H), 3.49–3.45 (m, 1H), 3.37 (t, $J = 10.9$ Hz, 1H), 2.79–2.70 (m, 1H), 2.35–2.25 (m, 1H), 1.48 (d, $J = 6.2$ Hz, 3H, Rha–CH_3), 1.43 (d, $J = 6.2$ Hz, 3H, Rha–CH_3), 0.97 (m, 6H, H-19, H-21), 0.78 (m, 6H, H-18, H-27); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 165.9, 165.8, 165.7, 165.3, 164.7, 140.6, 133.4–127.6 (36C), 121.7, 109.3, 97.0, 96.9, 96.4, 80.8, 80.5, 73.5, 72.4, 72.3, 71.7, 71.4, 71.3 (2C), 70.8, 70.6, 70.5, 68.0, 67.0, 66.9, 41.6, 40.3, 39.8, 38.4, 37.3, 37.0, 32.1, 31.9, 31.5, 31.4, 30.3, 29.8, 29.7 (3C), 28.8, 20.8, 19.3, 17.8, 17.7, 17.1, 16.2, 14.5, 14.1; MALDITOF MS: calcd for $C_{87}H_{94}O_{21}Na$ ($M+Na^+$), 1497.6180. Found: 1497.6164.

3.9. Diosgenyl 3,6-anhydro-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (30)

To a solution of **29** (80 mg, 0.054 mmol) in MeOH (2 mL) and CH_2Cl_2 (2 mL) was added NaOMe portionwise until the pH of the solution was between 10–11. After stirring for 1 h at room temperature, the reaction mixture was neutralized, filtered, and then concentrated. The residue was purified by silica gel column chromatography (5:1 CH_2Cl_2–MeOH) to afford **30** (20 mg, 43%) as a white solid: $[\alpha]_D^{23}$ −95.6 (*c* 0.74, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5): δ 5.70–5.64 (m, 3H, H-1′, Rha–H-1), 5.36 (br s, 1H, H-6), 4.80–4.33 (m, 13H), 4.05 (dd, $J = 3.0$, 10.0 Hz, 1H), 3.95–3.85 (m, 1H), 3.68–3.59 (m, 2H), 2.82–2.77 (m, 1H), 2.54–2.51 (m, 1H), 1.75 (d, $J = 6.1$ Hz, 3H, Rha–CH_3), 1.71 (d, $J = 6.1$ Hz, 3H, Rha–CH_3), 1.23 (d, $J = 6.9$ Hz, 3H, H-21), 1.07 (s, 3H, H-19), 0.92 (s, 3H, H-18), 0.78 (d, $J = 5.6$ Hz, 3H, H-27); ^{13}C NMR (100 MHz, pyridine-d_5): δ 141.0, 121.8, 109.4, 101.5, 101.1, 98.1, 81.2 (2C), 77.4, 74.1, 73.9, 73.8, 73.2, 72.7 (2C), 72.5 (2C), 72.3, 71.5, 70.5, 70.2, 67.0, 63.1, 56.8, 50.5, 42.1, 40.6, 40.0, 39.1, 37.7, 37.3, 32.4, 32.3, 32.0, 31.8, 30.7, 30.4, 29.4, 21.3, 19.5, 18.7, 18.6, 17.4, 16.5, 15.1; MALDITOF MS: calcd for $C_{45}H_{70}O_{15}Na$ ($M+Na^+$), 873.5. Found: 873.4.

3.10. Diosgenyl 3,6-anhydro-2-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (31)

To a mixture of compound **27** (116 mg, 0.14 mmol), imidate **19** (178 mg, 1.4 mmol), and 4 Å MS in anhyd CH_2Cl_2 (5 mL) at 0 °C under N_2 was added TMSOTf (2.4 μL, 0.014 mmol). After stirring for 0.5 h, the mixture was allowed to warm up to room temperature and was stirred for another 4 h. The reaction was then quenched with Et_3N. The resulting mixture was then filtered and concentrated. The residue was applied to silica gel column chromatography (30:1 petroleum ether–EtOAc) to provide **31** (113 mg, 65%) as a white solid: $[\alpha]_D^{23}$ +7.3 (*c* 1.37, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, $J = 7.5$ Hz, 2H, Ar–H), 7.92 (d, $J = 7.5$ Hz, 2H, Ar–H), 7.83–7.78 (m, 6H, Ar–H), 7.65–7.16 (m, 15H, Ar–H), 5.87–5.84 (m, 1H), 5.75–5.65 (m, 3H), 5.42 (br s, 1H, H-6), 5.20 (s, 1H, H-1′), 4.57–4.52 (m, 1H), 4.45–4.40 (m, 1H), 4.14 (br s, 2H), 3.97 (d, $J = 4.8$ Hz, 1H), 3.91–3.84 (m, 2H), 3.73–3.66 (m, 2H), 3.51–3.47 (m, 1H), 3.43–3.36 (m, 1H), 2.64–2.60 (m, 1H), 2.44–2.26 (m, 3H), 1.37 (d, $J = 5.1$Hz, 3H, Rha–CH_3), 1.22 (s, 9H, TBDPS–$(CH_3)_3$), 0.98 (d, $J = 6.9$ Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.79 (br s, 6H, H-18, H-27); ^{13}C NMR (75 MHz, $CDCl_3$): δ

165.8, 165.6, 165.0, 140.6, 135.8–127.8 (30C), 121.7, 109.3, 95.7, 94.8, 80.8, 80.1, 78.4, 75.3, 73.7, 73.0, 72.8, 72.1, 71.0, 69.7, 66.9, 66.6, 62.2, 56.5, 50.1, 41.7, 40.3, 39.8, 39.1, 37.3, 37.0, 32.2, 31.9, 31.6, 31.4, 30.3, 30.0, 28.8, 26.8 (3C), 20.9, 19.3, 19.2, 17.5, 17.1, 16.3, 14.5; MALDITOF MS: calcd for $C_{76}H_{90}O_{14}SiNa$ ($M+Na^+$), 1277.5992. Found: 1277.5982.

3.11. Diosgenyl 3,6-anhydro-4-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (32)

To a solution of **31** (40 mg, 0.032 mmol) in THF (1 mL) was added TBAF (1 mmol). The solution was neutralized with AcOH and stirred at room temperature for 6 h. MeOH (2 mL) and NaOMe was added to adjust the pH $\geqslant$9. The resulting solution was stirred for another 2 h and then concentrated. The residue was purified by silica gel column chromatography (20:1 CH_2Cl_2–MeOH) to afford a crude oil, which was then washed with water to provide **32** (21 mg, 94%) as a white solid: $[\alpha]_D^{22}$ −113.7 (*c* 0.51, $CHCl_3$); ^{1}H NMR (300 MHz, pyridine-d_5): δ 5.84 (s, 1H, Rha–H-1), 5.65 (s, 1H, H-1′), 5.26 (br s, 1H, H-6), 5.06 (br s, 8H), 4.60–4.51 (m, 8H), 4.41–4.30 (m, 2H), 4.01–3.91 (m, 2H), 3.61–3.48 (m, 2H), 2.77–2.73 (m, 1H), 2.56–2.49 (m, 1H), 2.17–2.14 (m, 1H), 1.71 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.15 (d, $J = 6.3$ Hz, 3H, H-21), 0.99 (s, 3H, H-19), 0.84 (s, 3H, H-18), 0.70 (br s, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.0, 121.9, 109.4, 100.8, 98.0, 81.2, 79.8, 77.5, 75.1, 74.0, 73.0, 72.8, 72.4, 72.3, 71.1, 70.5, 67.0, 63.1, 56.8, 50.5, 42.1, 40.6, 40.0, 39.2, 37.8, 37.3, 32.4, 32.3, 32.0, 31.0, 30.7, 30.4, 29.4, 21.3, 19.5, 18.7, 17.4, 16.5, 15.2; MALDITOF MS: calcd for $C_{39}H_{60}O_{11}Na$ ($M+Na^+$), 727.4028. Found: 727.4044.

3.12. Diosgenyl 6-*O*-ethyl-β-D-glucopyranoside (33)

To a solution of compound **25** (100 mg, 0.14 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 3.8 mg, 0.015 mmol) in anhyd CH_2Cl_2 (1 mL), ethyl vinyl ether (0.08 mL, 0.8 mmol) was added dropwise under N_2. After stirring overnight, the mixture was poured slowly into cold satd aq $NaHCO_3$ (2 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with satd aq NaCl (2 × 10 mL) and dried over $MgSO_4$. The solvent was removed by rotary evaporation to afford a yellow solid that was added to a stirred solution of Na (52 mg, 2.3 mmol) in EtOH (3 mL, 50 mmol). The reaction was held at reflux for 8 h, and then cooled to room temperature and concentrated. The residue was dissolved in EtOAc (25 mL) and H_2O (30 mL). The aq layer was extracted with EtOAc (3 × 25 mL), and the combined organic layers were washed with satd aq NaCl, dried over $MgSO_4$, and concentrated by rotary evaporation. The resulting oil was added to 50% AcOH (5 mL). After stirring overnight, water was added. The resulting mixture was concentrated by rotary evaporation to give a residue that was subjected to silica gel column chromatography (30:1 CH_2Cl_2–MeOH) to provide **33** (55 mg, 67%) as a white solid: $[\alpha]_D^{27}$ −98.0 (*c* 0.55, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 5.31 (br s, 1H, H-6), 4.98 (d, $J = 7.5$ Hz, 1H, H-1′), 4.55 (q, $J = 7.5$ Hz, 1H, H-16), 4.29–4.20 (m, 2H), 4.15–3.89 (m, 6H), 3.61–3.50 (m, 4H), 2.72–2.67 (m, 1H), 2.47–2.39 (m, 1H), 1.15 (t, $J = 6.9$ Hz, 3H, Et–CH_3), 1.14 (d, $J = 6.9$ Hz, 3H, H-21), 0.90 (s, 3H, H-19), 0.83 (s, 3H, H-18), 0.70 (d, $J = 5.4$ Hz, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.1, 121.8, 109.4, 102.8, 81.2, 78.7, 78.4, 77.1, 75.4, 71.8, 71.4, 67.1, 67.0, 63.1, 56.8, 50.4, 42.1, 40.6, 40.0, 39.5, 37.6, 37.2, 32.4, 32.3, 32.0, 31.8, 30.7, 30.4, 29.4, 21.3, 19.5, 17.5, 16.5, 15.7, 15.2; HRESIMS: calcd for $C_{35}H_{57}O_8$ ($M+H^+$), 605.4053. Found: 605.4072.

3.13. Diosgenyl 6-*O*-allyl-β-D-glucopyranoside (34)

A similar procedure used for the preparation of **33** was employed. Thus, treatment of compound **25** (100 mg, 0.14 mmol) through three steps provided **34** (60 mg, 71%) as a white solid: $[\alpha]_D^{27}$ −93.6 (*c* 0.52, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.01–5.91 (m, 1H, All–CH), 5.35–5.30 (m, 2H, H-6, All–C=CH_2), 5.10 (d, $J = 10.5$ Hz, 1H, All–C=CH_2), 4.96 (d, $J = 7.5$ Hz, 1H, H-1′), 4.52 (q, $J = 7.8$Hz, 1H, H-16), 4.26–4.21 (m, 2H), 4.14–3.91 (m, 7H), 3.59–3.44 (m, 2H), 2.67 (m, 1H), 2.42 (m, 1H), 1.12 (d, $J = 6.6$ Hz, 3H, H-21), 0.88 (s, 3H, H-19), 0.81 (s, 3H, H-18), 0.68 (d, $J = 3.9$ Hz, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.1, 136.1, 121.8, 116.2, 109.4, 102.8, 81.2, 78.7, 78.5, 77.2, 75.3, 72.6, 71.7, 71.1, 67.0, 63.0, 56.8, 50.4, 42.1, 40.6, 40.0, 39.5, 37.6, 37.2, 32.4, 32.3, 32.0, 31.8, 30.7, 30.4, 29.4, 21.3, 19.5, 17.4, 16.5, 15.1; HRESIMS: calcd for $C_{36}H_{57}O_8$ ($M+H^+$), 617.4053. Found: 617.4060.

3.14. Diosgenyl 6-*O*-(2-azidoethyl)-β-D-glucopyranoside (35)

A similar procedure used for the preparation of **33** was employed. Thus, treatment of compound **25** (755 mg, 1.03 mmol) through three steps provided **35** (372 mg, 56%) as a pale-yellow solid: $[\alpha]_D^{27}$ −85.2 (*c* 0.50, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 5.34 (br s, 1H, H-6), 4.98 (m, 1H, H-1′), 4.57 (br s, 1H, H-16), 4.32–4.21 (m, 2H), 4.06–3.99 (m, 5H), 3.78 (q, $J = 4.8$ Hz, 2H), 3.61–3.34 (m, 5H), 2.73–2.68 (m, 1H), 2.49–2.41 (m, 1H), 0.70 (br s, 3H); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.1, 121.8, 109.4, 102.8, 81.2, 78.7, 78.6, 77.1, 75.3, 72.0, 71.7, 70.8, 67.0, 63.0, 56.8, 51.2, 50.4, 42.1, 40.6, 40.0, 39.5, 37.7, 37.2, 32.4, 32.3, 32.0, 31.8, 30.7, 30.4, 29.4, 21.3, 19.5, 17.5, 16.5,

15.2; HRESIMS: calcd for $C_{35}H_{56}N_3O_8$ ($M+H^+$), 646.4067. Found: 646.4018.

3.15. Diosgenyl 6-*O*-(2-azidoethyl)-3-*O*-pivaloyl-β-D-glucopyranoside (36)

To a solution of triol **35** (1.21 g, 1.87 mmol) in anhyd CH_2Cl_2 (4 mL) and pyridine (8 mL) at −10 °C was added pivaloyl chloride (0.69 mL, 5.6 mmol) in anhyd CH_2Cl_2 (4 mL) portionwise under N_2. The mixture was warmed to −4 °C and stirred for 4.5 h, and was then quenched with MeOH and concentrated. The resulting residue was dissolved in EtOAc (100 mL) and washed with 5% HCl (2 × 70 mL) and satd aq NaCl. The organic layer was dried over $MgSO_4$ and concentrated to give a residue that was purified by silica gel column chromatography (8:1 petroleum ether–EtOAc) to provide **35** (1.04 g, 76%) as a white solid: $[\alpha]_D^{27}$ −65.5 (*c* 0.56, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.29 (br s, 1H, H-6), 4.81 (t, $J = 9.6$ Hz, 1H, H-3′), 4.39–4.33 (m, 2H, H-16, H-1′), 3.74–3.27 (m, 15H), 2.37–2.14 (m, 7H), 1.17 (s, 9H, Piv), 0.95 (s, 3H, H-19), 0.90 (d, $J = 9.9$ Hz, 3H, H-21). 0.73–0.72 (m, 6H, H-18, H-27); HRESIMS: calcd for $C_{40}H_{64}N_3O_9$ ($M+H^+$), 730.4643. Found: 730.4642.

3.16. Diosgenyl 6-*O*-ethyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (2)

3.16.1. Diosgenyl 6-*O*-ethyl-3-*O*-pivaloyl-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (21). A similar procedure used for the preparation of **20** was employed. Thus, treatment of **16** (263 mg, 0.38 mmol) with imidate **19** (1.41 g, 2.28 mmol) in the presence of TMSOTf (7 μL, 0.04 mmol) afforded **21** (0.59 g, 97%) as a pale-yellow solid: $[\alpha]_D^{27}$ +63.4 (*c* 0.48, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, $J = 7.2$ Hz, 4H, Ar–H), 8.00 (d, $J = 6.9$ Hz, 2H, Ar–H), 7.94 (d, $J = 9.0$ Hz, 2H, Ar–H), 7.85 (d, $J = 8.4$ Hz, 2H, Ar–H), 7.77 (d, $J = 8.7$ Hz, 2H, Ar–H), 7.65–7.18 (m, 18H, Ar–H), 5.83–5.49 (m, 7H), 5.14 (br s, 2H), 4.77–4.75 (m, 2H), 4.49–4.42 (m, 1H), 4.34–4.29 (m, 1H), 4.13–4.06 (m, 1H), 4.02–3.98 (m, 1H), 3.86–3.64 (m, 5H), 3.54–3.38 (m, 2H), 2.66–2.61 (m, 2H), 1.17 (s, 9H, Piv), 1.01 (d, $J = 6.6$ Hz, 3H, H-21), 0.95 (s, 3H, H-19), 0.82 (m, 6H, H-18, H-27).

3.16.2. Diosgenyl 6-*O*-ethyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (2). A similar procedure used for the preparation of **1** was employed. Thus treatment of **21** (160 mg, 0.10 mmol) with $LiOH{\cdot}H_2O$ (292 mg, 6.95 mmol) afforded **2** (74 mg, 83%) as a white solid: $[\alpha]_D^{27}$ −105.2 (*c* 0.52, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.39 (s, 1H, Rha–H-1), 5.59 (s, 1H, Rha–H-1), 5.31 (br s, 1H, H-6), 4.95–4.82 (m, 5H), 4.54–4.49 (m, 4H), 4.39–4.29 (m, 2H), 4.22–4.19 (m, 3H), 3.90–3.79 (m, 3H), 3.67–3.40 (m, 5H), 2.82–2.68 (m, 2H), 1.76 (d, $J = 5.7$ Hz, 3H, Rha–CH_3), 1.63 (d, $J = 6.6$ Hz, 3H, Rha–CH_3), 1.14 (d, $J = 7.5$ Hz, 3H, H-21), 1.11 (t, $J = 6.9$ Hz, 3H, Et–CH_3), 1.03 (s, 3H, H-19), 0.82 (s, 3H, H-18), 0.70 (d, $J = 5.4$ Hz, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.0, 121.9, 109.4, 103.0, 102.2, 100.6, 81.2, 79.3, 78.5, 78.0, 77.8, 75.5, 74.3, 74.0, 73.0, 72.9, 72.7, 72.6, 70.6, 70.0, 69.7, 67.0 (2C), 63.1, 56.8, 50.5, 42.1, 40.6, 40.0, 39.2, 37.7, 37.3, 32.5, 32.4, 32.0, 31.8, 30.7, 30.3, 29.4, 21.2, 19.5, 18.7, 18.6, 17.4, 16.5, 15.5, 15.1; HRESIMS: calcd for $C_{47}H_{76}O_{16}Na$ ($M+Na^+$), 919.5031. Found: 919.5015.

3.17. Diosgenyl 6-*O*-allyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (3)

3.17.1. Diosgenyl 6-*O*-allyl-3-*O*-pivaloyl-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (22). A similar procedure used for the preparation of **20** was employed. Thus, treatment of **17** (317 mg, 0.45 mmol) with imidate **19** (1.68 g, 2.70 mmol) in the presence of TMSOTf (8 μL, 0.04 mmol) afforded **22** (691 mg, 95%) as a pale-yellow solid: $[\alpha]_D^{27}$ +65.7 (*c* 0.52, $CHCl_3$–MeOH 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (d, $J = 7.5$ Hz, 4H, Ar–H), 8.00 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.94 (d, $J = 6.9$ Hz, 2H, Ar–H), 7.85 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.77 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.75–7.21 (m, 18H, Ar–H), 6.03–5.94 (m, 1H), 5.83–5.15 (m, 15H), 4.81–4.76 (m, 2H), 4.50–4.42 (m, 1H), 4.33–4.28 (m, 1H), 4.19–3.71 (m, 7H), 3.53–3.38 (m, 2H), 2.66–2.61 (m, 1H), 2.48–2.44 (m, 1H), 1.17 (s, 9H, Piv), 1.01 (d, $J = 7.2$Hz, 3H, H-21), 0.96 (s, 3H, H-19), 0.82 (m, 6H, H-18, H-27).

3.17.2. Diosgenyl 6-*O*-allyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (3). A similar procedure used for the preparation of **1** was employed. Thus treatment of **22** (168 mg, 0.10 mmol)with $LiOH{\cdot}H_2O$ (250 mg, 6.00 mol) afforded **3** (72 mg, 79%) as a white solid: $[\alpha]_D^{27}$ −104.3 (*c* 0.51, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.40 (s, 1H, Rha–H-1), 5.96–5.85 (m, 1H, All–CH), 5.61 (s, 1H, Rha–H-1), 5.32–5.25 (m, 2H), 5.10–4.82 (m, 9H), 4.65–4.49 (m, 4H), 4.39–4.29 (m, 2H), 4.24–4.19 (m, 3H), 4.02–3.84 (m, 5H), 3.70–3.50 (m, 4H), 2.81–2.67 (m, 2H), 1.76 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.62 (d, $J = 6.3$ Hz, 3H, Rha–CH_3), 1.14 (d, $J = 7.2$ Hz, 3H, H-21), 1.03 (s, 3H, H-19), 0.82 (s, 3H, H-18), 0.69 (d, $J = 5.7$ Hz, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.0, 136.0, 121.9, 116.8, 109.4, 103.1, 102.2, 100.6, 81.2, 79.4, 78.6, 78.0, 77.9, 75.5, 74.3, 74.0, 72.9, 72.8, 72.7, 72.6, 72.5, 70.7, 69.7, 69.6, 67.0, 63.1, 56.8, 50.5, 42.1, 40.6, 40.0, 39.2, 37.7, 37.3, 32.5, 32.4, 32.0, 31.8, 30.7, 30.3, 29.4, 21.2, 19.5, 18.8, 18.6, 17.5, 16.5, 15.2;

HRESIMS: calcd for $C_{48}H_{77}O_{16}$ ($M+H^+$), 909.5212. Found: 909.5209.

3.18. Diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-6-*O*-benzyl-β-D-glucopyranoside (4)

3.18.1. Diosgenyl 6-*O*-benzyl-3-*O*-pivaloyl-β-D-glucopyranoside (18). The crude compound **13** prepared from diosgenyl 4,6-*O*-benzylidene-β-D-glucopyranoside was dissolved in anhyd CH_2Cl_2 (5 mL). The solution was cooled to 0 °C, and Et_3SiH (0.51 mL, 3.2 mmol) and $BF_3{\cdot}Et_2O$ (0.1 mL, 0.8 mmol) were added under N_2. After stirring at room temperature for 1.5 h, the resulting mixture was diluted with EtOAc (50 mL) and washed with satd aq $NaHCO_3$. The organic layer was dried with Na_2SO_4 and was then concentrated. The residue was subjected to silica gel column chromatography (7:1 petroleum ether–EtOAc) to provide **18** (157 mg, 19%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (br s, 5H, Ar–H), 5.36 (br s, 1H, H-6), 4.91–4.86 (m, 1H, H-3′), 4.60 (br s, 2H), 4.46–4.41 (m, 2H), 3.78–3.35 (m, 8H), 2.40–2.23 (m, 2H), 1.25 (s, 9H, Piv), 1.03 (s, 3H, H-19), 0.98 (d, $J = 6.6$ Hz, 3H, H-21), 0.80 (br s, 6H, H-18, H-27); MALDITOF MS: calcd for $C_{45}H_{66}O_9Na$ ($M+Na^+$), 773.5. Found: 773.5.

3.18.2. Diosgenyl 6-*O*-benzyl-3-*O*-pivaloyl-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (23). A similar procedure used for the preparation of **20** was employed. Thus, treatment of **18** (159 mg, 0.21 mmol) with imidate **19** (789 mg, 1.26 mmol) in the presence of TMSOTf (4 μL, 0.02 mmol) afforded **23** (289 mg, 80%) as a white solid: $[\alpha]_D^{28}$ +68.6 (*c* 0.51, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (d, $J = 7.4$ Hz, 4H, Ar–H), 7.98 (d, $J = 7.2$ Hz, 2H, Ar–H), 7.92 (d, $J = 7.5$ Hz, 2H, Ar–H), 7.85 (d, $J = 7.4$ Hz, 2H, Ar–H), 7.74 (d, $J = 7.1$ Hz, 2H, Ar–H), 7.62–7.58 (m, 2H, Ar–H), 7.50–7.19 (m, 21H, Ar–H), 5.81–5.46 (m, 8H), 5.14 (br s, 2H), 4.76–4.66 (m, 4H), 4.45–4.43 (m, 1H), 4.29–4.24 (m, 1H), 4.16–3.68(m, 7H), 3.51–3.41 (m, 2H), 2.35–2.26 (m, 2H), 1.15 (s, 9H, Piv), 0.99 (d, $J = 6.5$ Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.79 (br s, 6H, H-18, H-27).

3.18.3. Diosgenyl 6-*O*-benzyl-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (4). A similar procedure used for the preparation of **1** was employed. Thus treatment of **23** (260 mg, 0.15 mmol) with $LiOH{\cdot}H_2O$ (393 mg, 9.4 mol) afforded **4** (101 mg, 70%) as a white solid: $[\alpha]_D^{28}$ −102.0 (*c* 0.53, MeOH); ^{1}H NMR (400 MHz, pyridine-d_5): δ 7.53 (d, $J = 7.09$ Hz, 2H, Ar–H), 7.43–7.40 (m, 2H, Ar–H), 7.35 (m, 1H, Ar–H), 6.45 (s, 1H, Rha–H-1), 5.73 (s, 1H, Rha–H-1), 5.41 (br s, 1H, H-6), 5.04–4.89 (m, 15H), 4.71–4.57 (m, 6H), 4.45–4.24 (m, 5H), 4.03–3.92 (m, 3H), 3.80 (m, 1H), 3.68–3.56 (m, 2H), 2.90–2.77 (m, 2H), 1.84 (d, $J = 6.2$ Hz, 3H, Rha–CH_3), 1.70 (d, $J = 6.2$ Hz, 3H, Rha–CH_3), 1.22 (d, $J = 6.9$ Hz, 3H, H-21), 1.12 (s, 3H, H-19), 0.91 (s, 3H, H-18), 0.78 (d, $J = 5.5$ Hz, 3H, H-27); ^{13}C NMR (100 MHz, pyridine-d_5): δ 141.0, 139.1, 128.8 (2C), 128.2 (2C), 127.9, 121.9, 109.4, 103.0, 102.2, 100.7, 81.2, 79.3, 78.7, 78.0, 77.9, 75.5, 74.3, 74.0, 73.6, 72.9, 72.8, 72.7, 72.6, 70.7, 69.8, 69.7, 67.0, 63.0, 56.8, 50.5, 42.1, 40.6, 40.0, 39.2, 37.6, 37.3, 32.4, 32.3, 32.0, 31.8, 30.7, 30.4, 29.4, 21.2, 19.5, 18.8, 18.6, 17.4, 16.5, 15.1; MALDITOF MS: calcd for $C_{52}H_{78}O_{16}Na$ ($M+Na^+$), 981.5. Found: 981.8.

3.19. Diosgenyl 6-*O*-(2-azidoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (5)

3.19.1. Diosgenyl 6-*O*-(2-azidoethyl)-3-*O*-pivaloyl-2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (37). A similar procedure used for the preparation of **20** was employed. Thus, treatment of **36** (1.04 g, 1.42 mmol) with imidate **19** (5.30 g, 8.53 mmol) under the promotion of TMSOTf (26 μL, 0.14 mmol) afforded **37** (1.96 g, 84%) as a white solid: $[\alpha]_D^{27}$ +68.0 (*c* 0.51, $CHCl_3$–MeOH 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, $J = 7.5$ Hz, 4H, Ar–H), 8.00 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.94 (d, $J = 8.1$ Hz, 2H, Ar–H), 7.85 (d, $J = 7.8$ Hz, 2H, Ar–H), 7.78 (d, $J = 7.5$ Hz, 2H, Ar–H), 7.64–7.22 (m, 18H, Ar–H), 5.84–5.49 (m, 6H), 5.15 (br s, 2H), 4.78–4.76 (m, 2H), 4.50–4.45 (m, 1H), 4.34–4.29 (m, 1H), 4.18–4.07 (m, 3H), 3.96–3.76 (m, 7H), 2.65–2.62 (m, 1H), 2.49–2.41 (m, 1H), 2.09–2.01 (m, 1H), 1.28 (s, 6H), 1.17 (s, 9H, Piv), 1.01 (d, $J = 6.6$ Hz, 3H, H-21), 0.96 (s, 3H, H-19), 0.82 (m, 6H, H-18, H-27).

3.19.2. Diosgenyl 6-*O*-(2-azidoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (5). A similar procedure used for the preparation of **1** was employed. Thus treatment of **37** (1.70 g, 1.03 mmol) with $LiOH{\cdot}H_2O$ (4.34 g, 103 mmol) afforded **5** (796 mg, 82%) as a white solid: $[\alpha]_D^{27}$ −90.8 (*c* 0.46, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.37 (s, 1H, Rha–H-1), 5.59 (s, 1H, Rha–H-1), 5.32 (br s, 1H, H-6), 4.98–4.83 (m, 6H), 4.64–4.48 (m, 5H), 4.39–4.13 m, 6H), 3.85–3.67 (m, 4H), 3.67–3.46 (m, 6H), 3.34–3.28 (m, 2H), 2.83–2.67 (m, 2H), 1.75 (d, $J = 6.6$ Hz, 3H, Rha–CH_3), 1.61 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.14 (d, $J = 7.2$ Hz, 3H, H-21), 1.04 (s, 3H, H-19), 0.82 (s, 3H, H-18), 0.70 (d, $J = 4.8$ Hz, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.0, 121.9, 109.4, 103.1, 102.2, 100.6, 81.2, 79.5, 78.6, 78.0, 77.9, 75.4, 74.2, 73.9, 72.9, 72.8, 72.6 (2C), 70.7 (2C), 69.7, 67.0 (2C), 63.1, 56.8, 51.1, 50.5, 42.1, 40.6, 40.0, 39.2, 37.7, 37.3, 32.5, 32.4, 32.0, 31.8, 30.7, 30.4, 29.4, 21.3, 19.5, 18.8,

18.6, 17.5, 16.5, 15.2; HRESIMS: calcd for $C_{47}H_{75}N_3O_{16}Na$ ($M+Na^+$), 960.5040. Found: 960.5005.

3.20. Diosgenyl 6-*O*-(2-aminoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (6)

To a solution of azide **5** (52 mg, 0.055 mmol) in THF (1.6 mL) and H_2O (0.4 mL) was added PPh_3 (29 mg, 0.11 mmol). The mixture was warmed up to 60 °C and stirred for 2 h. The solvent was removed by rotary evaporation to give a white solid that was employed directly in the next acylation step. Silica gel column chromatography (4:1 CH_2Cl_2–MeOH) of the crude solid afforded **6** (48 mg, 94%) as a pale-yellow solid: $[\alpha]_D^{23}$ −72.2 (*c* 0.50, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5): δ 6.30 (s, 1H, Rha–H-1), 6.10 (s, 2H, NH_2), 5.63 (s, 1H, Rha–H-1), 5.32 (br s, 1H, H-6), 4.98–4.77 (m, 4H), 4.67–4.49 (m, 4H), 4.39–4.28 (m, 2H), 4.18–4.07 (m, 3H), 3.92–3.81 (m, 4H), 3.70–3.42 (m, 11H), 3.13 (br s, 1H), 2.89–2.62 (m, 2H), 1.75 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.58 (d, $J = 3.7$ Hz, 3H, Rha–CH_3), 1.12 (d, $J = 6.9$ Hz, 3H, H-21), 1.02 (s, 3H, H-19), 0.81 (s, 3H, H-18), 0.68 (d, $J = 4.8$ Hz, 3H, H-27); HRESIMS: calcd for $C_{47}H_{78}NO_{16}$ ($M+H^+$), 912.5321. Found: 912.5328.

3.21. Diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-6-*O*-(2-acetylaminoethyl)-β-D-glucopyranoside (7)

To a stirred solution of crude **6**, prepared from azide **5** (0.032 mmol), and Et_3N (27 μL, 0.19 mmol) in MeOH (2 mL) at 0 °C was added AcCl (11 μL, 0.15 mmol). The solution was warmed to room temperature and stirred for 2 h. The mixture was concentrated to give a residue that was purified by silica gel column chromatography (12:1 CH_2Cl_2–MeOH) to afford **7** (25 mg, 82% based on **5**) as a white solid: $[\alpha]_D^{23}$ −70.2 (*c* 0.47, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5): δ 6.33 (s, 1H, Rha–H-1), 5.53 (s, 1H, Rha–H-1), 5.29 (br s, 1H, H-6), 4.86–4.79 (m, 5H), 4.61–4.13 (m, 8H), 3.86–3.77 (m, 2H), 3.62–3.43 (m, 6H), 2.09 (s, 3H), 1.75 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.59 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.12 (d, $J = 6.9$ Hz, 3H, H-21), 1.01 (s, 3H, H-19), 0.80 (s, 3H, H-18), 0.67 (d, $J = 5.2$ Hz, 3H, H-27); HRESIMS: calcd for $C_{49}H_{80}NO_{17}$ ($M+H^+$), 954.5426. Found: 954.5473.

3.22. Diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-6-*O*-(2-trifluoroacetylaminoethyl)-β-D-glucopyranoside (8)

A similar procedure used for the preparation of **7** was employed. Thus treatment of crude **6** (prepared from 0.028 mmol **5**) with $(CF_3CO)_2O$ (36 μL, 0.26 mmol) afforded **8** (25 mg, 90% based on **5**) as a pale-yellow solid: $[\alpha]_D^{22}$ −81.0 (*c* 0.49, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5): δ 11.01 (s, 1H, NH), 6.36 (s, 1H, Rha–H-1), 5.50 (s, 1H, Rha–H-1), 5.29 (br s, 1H, H-6), 4.98–4.81 (m, 4H), 4.63–4.62 (m, 3H), 4.41–4.30 (m, 2H), 4.13 (br s, 2H), 3.86–3.49 (m, 8H), 2.75–2.71 (m, 2H), 1.78 (d, $J = 6.0$ Hz, 3H, Rha–CH_3), 1.13 (d, $J = 6.6$ Hz, 3H, H-21), 1.02 (s, 3H, H-19), 0.81 (s, 3H, H-18), 0.68 (br s, 3H, H-27); HRESIMS: calcd for $C_{49}H_{77}F_3NO_{17}$ ($M+H^+$), 1008.5144. Found: 1008.5113.

3.23. Diosgenyl 6-*O*-(2-benzoylaminoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (9)

A similar procedure used for the preparation of **7** was employed. Thus treatment of the crude **6** (prepared from 0.077 mmol **5**) with benzoyl chloride (45 μL, 0.39 mmol) afford **9** (75 mg, 96% based on **5**) as a white solid: $[\alpha]_D^{23}$ −90.0 (*c* 0.53, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5): δ 9.17 (s, 1H, NH), 8.30 (m, 2H, Ar–H), 7.43–7.41 (m, 3H, Ar–H), 6.34 (s, 1H, Rha–H-1), 5.59 (s, 1H, Rha–H-1), 5.29 (br s, 1H, H-6), 4.92–4.81 (m, 4H), 4.61–4.47 (m, 3H), 4.43–4.15 (m, 4H), 3.84–3.76 (m, 6H), 3.64–3.56 (m, 2H), 2.74 (br s, 1H), 2.79–2.63 (m, 2H), 0.99 (s, 3H, H-19), 0.80 (s, 3H, H-18), 0.69 (br s, 3H, H-27); ^{13}C NMR (75 MHz, pyridine-d_5): δ 168.1, 141.0, 131.5, 128.9 (2C), 128.3 (2C), 121.9, 109.4, 102.9, 102.3, 100.7, 81.3, 78.9, 78.7, 78.0, 77.9, 75.3, 74.2, 73.9, 73.0, 72.8, 72.6, 71.0, 70.7, 70.2, 69.7, 67.0, 63.1, 56.8, 50.5, 46.1, 42.1, 40.6, 40.4, 40.0, 39.2, 37.6, 37.3, 32.5, 32.4, 32.0, 31.8, 30.8, 30.3, 30.1, 29.4, 21.3, 19.5, 18.8, 18.6, 17.5, 16.5, 15.2; HRESIMS: calcd for $C_{54}H_{82}NO_{17}$ ($M+H^+$), 1016.5583. Found: 1016.5537.

3.24. Diosgenyl 6-*O*-(2-*N*-(2*E*,4*E*)-hexa-2,4-dienoylaminoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (10)

To a solution of sorbic acid (35 mg, 0.31 mmol) in anhyd CH_2Cl_2 (3 mL) were added oxalyl dichloride (53 μL, 0.62 mmol) and DMF (2 μL, 0.026 mmol). The solution was stirred for 3 h at room temperature and concentrated to afford a red oil that was added to a solution of crude **6** (prepared from 0.031 mmol **5**) and Et_3N (52 μL, 0.37 mmol) in MeOH (2.5 mL). The solvent was removed by rotary evaporation after stirring for 2.5 h. Silica gel column chromatography (10:1 CH_2Cl_2–MeOH) of the residue afforded **10** (29 mg, 93% based on **5**) as a white solid: $[\alpha]_D^{22}$ −91.9 (*c* 0.49, 1:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5): δ 8.88 (br s, 1H, NH), 7.62–7.54 (m, 1H), 6.37–6.31 (m, 2H), 6.15–6.07 (m, 1H), 5.92–5.85 (m, 1H), 5.57 (s, 1H, Rha–H-1), 5.29 (br s, 1H, H-6), 4.98–4.82 (m, 3H), 4.63–4.48 (m, 4H), 4.40–4.29 (m, 2H), 4.18 (m, 2H), 3.84–3.49 (m, 8H), 2.77–2.63 (m, 2H), 1.78 (d, $J = 6.3$ Hz, 3H), 1.61 (d, $J = 6.3$ Hz, 3H), 1.57 (d,

$J = 6.4$ Hz, 3H), 1.13 (d, $J = 6.6$ Hz, 3H, H-21), 1.02 (s, 3H, H-19), 0.81 (s, 3H, H-18), 0.68 (d, $J = 3.6$ Hz, 3H, H-27); HRESIMS: calcd for $C_{53}H_{84}NO_{17}$ ($M+H^+$), 1006.5739. Found: 1006.5678.

3.25. Diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-6-*O*-(2-tetradecanoylaminoethyl)-β-D-glucopyranoside (11)

To a solution of tetradecanoic acid (63 mg, 0.27 mmol) in anhyd CH_2Cl_2 (3 mL) were added oxalyl chloride (47 μL, 0.55 mmol) and DMF (2 μL, 0.026 mmol). The solution was stirred for 3 h at room temperature and concentrated to afford a yellow oil that was added to a solution of crude **6** (prepared from 0.027 mmol **5**) and Et_3N (47 μL, 0.34 mmol) in MeOH (2.5 mL). The solvent was removed by rotary evaporation after stirring for 2.5 h. Silica gel column chromatography (12:1 CH_2Cl_2–MeOH) afforded **11** (27 mg, 87% based on **5**) as a white solid: $[\alpha]_D^{23}$ −69.2 (*c* 0.50, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 8.63 (br s, 1H, NH), 6.34 (s, 1H, Rha–H-1), 5.54 (s, 1H, Rha–H-1), 5.29 (br s, 1H, H-6), 4.87–4.79 (m, 4H), 4.61–4.57 (m, 4H), 4.18–4.11 (m, 3H), 3.84–3.81 (m, 3H), 3.68–3.40 (m, 7H), 2.85 (q, $J = 7.2$ Hz, 4H), 2.49–2.39 (m, 2H), 1.75 (d, $J = 6.6$ Hz, 3H, Rha–CH_3), 1.60 (d, $J = 6.3$ Hz, 3H, Rha–CH_3), 1.29–1.11 (m, 28H), 1.02 (s, 3H), 0.86–0.80 (m, 6H), 0.68 (br s, 3H); HRESIMS: calcd for $C_{61}H_{104}NO_{17}$ ($M+H^+$), 1122.7304. Found: 1122.7312.

3.26. Diosgenyl 6-*O*-(dansylaminoethyl)-2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (12)

To a mixture of crude **6** (prepared from 0.053 mmol **5**) and $NaHCO_3$ (42 mg, 0.50 mmol) in MeOH (5 mL) was added dansyl chloride (99 mg, 0.37 mmol). After stirring for 24 h in darkness, the mixture was concentrated. The residue was purified by silica gel column chromatography (14:1 CH_2Cl_2–MeOH) to afford **12** (47 mg, 77% based on **5**) as a green solid: $[\alpha]_D^{23}$ −83.0 (*c* 0.52, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ9.76 (m, 1H, NH), 8.96 (d, $J = 8.8$ Hz, 1H, Dansyl), 8.58 (m, 2H, Dansyl), 7.64–7.52 (m, 2H, Dansyl), 7.16 (d, $J = 7.4$Hz, 1 H, Dansyl), 6.30 (s, 1H, Rha–H-1), 5.46 (s, 1H, Rha–H-1), 5.31 (br s, 1H, H-6), 4.97–4.75 (m, 5H), 4.62–4.50 (m, 3H), 4.45 (dd, $J = 4.0$, 9.1 Hz, 1H), 4.38–4.00 (m, 4H), 3.84–3.36 (m, 9H), 2.73 (s, 6H, Dansyl-$(CH_3)_2$), 1.78 (d, $J = 3.6$ Hz, 3H, Rha–CH_3), 1.12 (d, $J = 6.9$ Hz, 3H, H-21), 1.02 (s, 3H, H-19), 0.81 (s, 3H, H-18), 0.68 (d, $J = 5.2$ Hz, 3H, H-27); HRESIMS: calcd for $C_{59}H_{89}N_2O_{18}S$ ($M+H^+$), 1145.5831. Found: 1145.5870.

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Supplementary data

Supplementary data (^{1}H NMR spectra of compounds **2**–**12**, **16**–**18**, **21**–**23**, **26**, **27**, and **29**–**37** and ^{13}C NMR spectra of compounds **26**, **27**, **29**–**35**, **2**–**5**, and **9**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.09.004.

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