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Synthesis of neosaponins and neoglycolipids containing a chacotriosyl moiety

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Abstract—α-L-Rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranose (chacotriose) is the oligosaccharide moiety of dioscin. Chacotriosyl trichloroacetimidate was synthesized from D-glucose and L-rhamnose, and glycosylated to mevalonate (diosgenin, cholesterol, and glycyrrhetic acid) to yield dioscin and neosaponins. In order to simplify the structure of the aglycone part, the mevalonate moiety was replaced with double-chain neoglycolipids that mimicked glycosyl ceramides. A cytotoxicity test revealed the importance of the glycosidic linkage of the naturally occurring β-form and that dioscin and the neoglycolipid with the longest chain showed a moderate activity.

Keywords: Solanaceous plant; Chacotriose; Glycoconjugate; Neosaponin; Neoglycolipid; Cytotoxicity

1. Introduction

Several studies have reported that steroidal saponins isolated from various natural products have pharmaceutical and biological activities.[1] We have studied steroidal oligosaccharides in many solanaceous plants and clarified some of their biological activities.[2] In particular, certain kinds of saponins from plants belonging to the genus *Solanum*, for example, *S. dulcamara*, *S. lyratum*, and *S. nigrum*, exhibited moderate to good anti-cancer[3] and anti-herpes[4] activities. The results showed that spirostane-type glycosides, particularly those containing chacotriose as an oligosaccharide moiety represented by dioscin,[5] were highly effective. On the other hand, other aglycone-type chacotriosyl glycosides had almost no activity or weak activities. In addition, Kuo and co-workers[6] reported that the chacotriosyl moiety of solamargine played a crucial role in triggering cell death by apoptosis. Therefore, we desired to investigate the structure–activity relationship (SAR) of the aglycone part of chacotriosyl glycosides. However, often only small amounts of these compounds are available from natural sources, and it is extremely difficult to obtain glycosides with the desired structure to attain a comparable SAR. Thus, the synthesis of chacotriosyl neoglycosides containing many types of aglycones from commercial, easily available starting materials is interesting. Although several researches on the synthesis of chacotriose analogues have been reported,[7] our synthesis method[8] may be useful for directly transferring the chacotriose moiety to various aglycones. In this paper, we have established a facile synthesis method of producing chacotriosyl donors by chemical and enzymatic reactions, and have transferred the chacotriosyl moiety to some mevalonates to afford neosaponins. Furthermore, to simplify the aglycone structure, we converted mevalonate to a double-chain lipid mimicking ceramide as the aglycone part to obtain some neoglycolipids containing chacotriose. Finally, we examined the cytotoxicity of the synthesized glycoconjugates.

2. Results and discussion

The chacotriosyl donor has 2 L-rhamnose units linked at the 2- and 4-hydroxy groups of D-glucose; the 1-, 3-, and

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6-hydroxy groups must be protected, and the anomeric group of the D-glucose unit must be reactivated for the transglycosylation to aglycone. The anomeric position of D-glucose was protected with allylalcohol, which can also serve as a scaffold for the C–C bond.

D-Glucose was enzymatically converted to allyl-β-D-glucopyranoside **1**[9] (45%) by using almond β-glucosidase and allylalcohol (Scheme 1). When compared with the conventional chemical[10] and enzymatic syntheses of **1**, the number of processes was decreased to one step, although the process yield was the same as that obtained by the chemical one. Next, the 3- and 6-hydroxy groups of **1** were selectively protected using pivaloyl chloride in pyridine at −15 °C for 2 h to afford allyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside **2**.[11] Glycosylation of glucopyranoside **2** with 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl trichloroacetimidate **3**[12] in the presence of boron trifluoride etherate ($BF_3{\cdot}Et_2O$) as a promoter provided the desired fully protected chacotrioside **4** (Table 1, entry 1). In this reaction, small amounts of undesirable 2- or 4-O-glycosylated disaccharides were obtained; hence, we attempted to compare the yields of chacotrioside **4**[13] by using the 'Normal Procedure' (N.P.) and Schmidt's 'Inverse Procedure' (I.P.) to determine, which procedure provided a greater yield. When I.P. was used instead of N.P., the yield of **4** increased remarkably (Table 1, entries 5 and 6). Moreover, the yield of glycosylation when using $BF_3{\cdot}Et_2O$ as a promoter (Table 1, entry 5) was better than that obtained using trimethylsilyl trifluoromethanesulfonate (TMSOTf) (Table 1, entry 6). The reason for the improved reaction yield might be the prevention of the decomposition of donor **3** during the glycosidic coupling reaction. The fully protected chacotrioside **4** was treated with tetrakis (triphenylphosphine) palladium in acetic acid to give trisaccharide **5** in 78% yield.[14] The anomeric hydroxy group of **5** was converted to an α-trichloroacetimidate derivative **6** (86%) in the presence of trichloroacetonitrile and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU).[15]

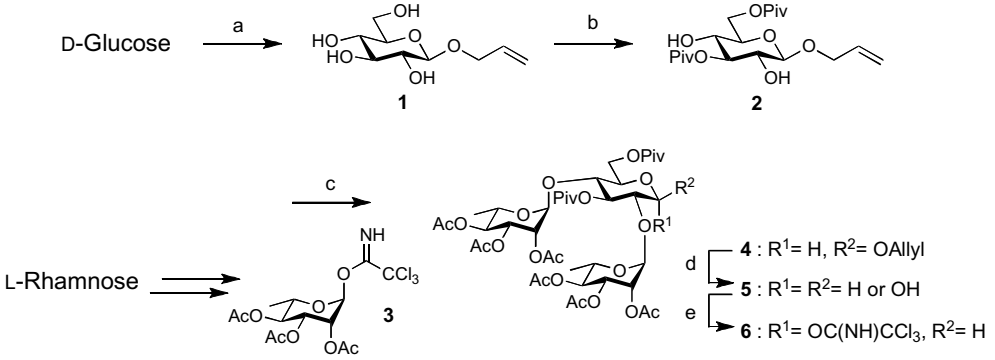
In order to investigate their SAR, chacotriosyl α-trichloroacetimidate **6** was transferred to several types of

Table 1. Glycosylation of **2** and **3** under various conditions

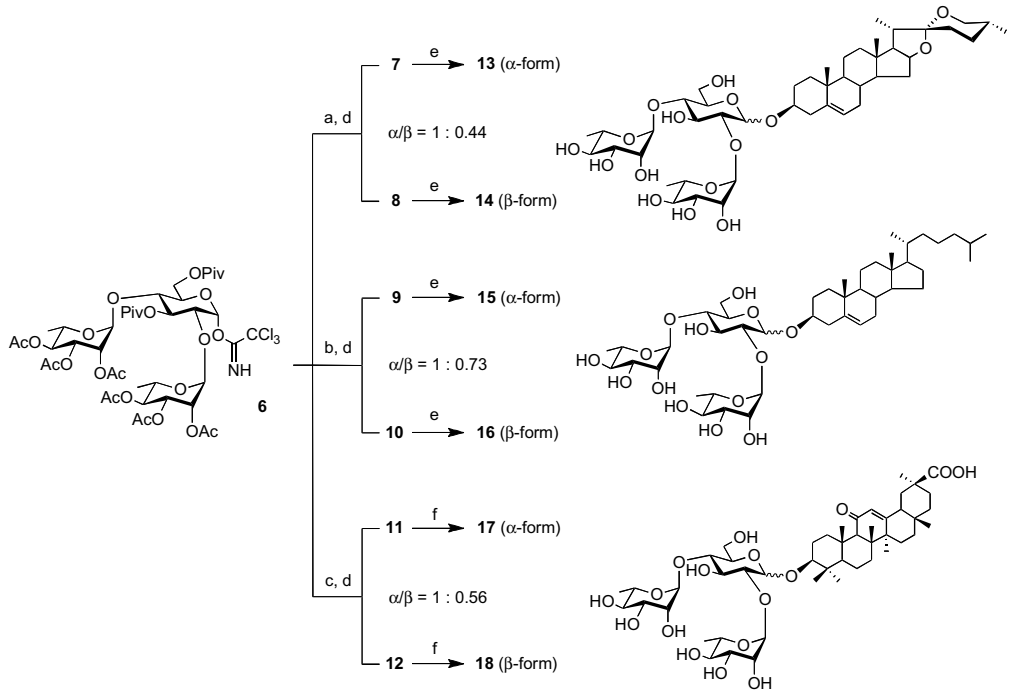
Entries	Method	Lewis acid (equiv)	Temp (°C)	Yield (%)
1	N.P.	$BF_3{\cdot}Et_2O$ (2.0)	0	41
2	N.P.	$BF_3{\cdot}Et_2O$ (4.3)	0	54
3	N.P.	TMSOTf (0.8)	0	46
4	N.P.	$BF_3{\cdot}Et_2O$ (3.8)	−78	65
5	I.P.	$BF_3{\cdot}Et_2O$ (4.4)	−78	84
6	I.P.	TMSOTf (1.0)	−78	60

I.P. = Inverse procedure, N.P. = normal procedure.

mevalonates (C_{27} steroid-type, cholestane-type, and triterpenoid-type skeletons) as an aglycone part. Initially, **6** was transferred to diosgenin as an aglycone part of dioscin in the presence of $BF_3{\cdot}Et_2O$ at 20 °C to afford protected neoglycosides (**7** and **8**) as a mixture of α/β anomers (α:β = 1:0.44) in 64% yield (Scheme 2). Although the stereoselectivity of this glycosylation was not sufficient to predominantly obtain the naturally occurring β anomer, the anomeric mixtures of the protected neoglycosides were easily separated using octadodecyl silica gel (ODS) column chromatography. In the same manner, **6** was transferred to the other aglycones (cholesterol and glycyrrhetic acid) at room temperature in the presence of $BF_3{\cdot}Et_2O$ to obtain protected neoglycosides **9** and **10** in 77% and **11** and **12** in 64% yields, respectively (Scheme 2). The obtained α/β anomeric mixture of protected neosaponins was separated using ODS column chromatography with 90% MeOH to yield pure α- and β-protected neoglycosides (**9**–**12**). Each protected neoglycoside (**7**–**12**) was deprotected in the usual manner to afford neosaponins (**13**–**18**), respectively, except for **14** (dioscin). It is noteworthy that α-chacotriosyl diosgenin cannot be obtained from a natural source; therefore, compound **13** should be a good candidate for comparison with dioscin in terms of glycosidic linkages and biological activities. On the other hand, the stepwise synthesis of β-chacotriosyl derivatives from the glycosylation of some steroids and glucose units was established;[7] the transglycosylation method of the chacotriosyl moiety described here was useful for preparing various types of aglycone parts



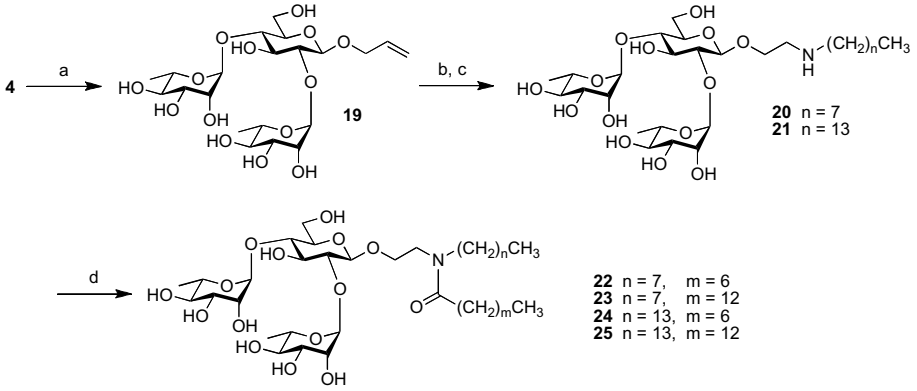
Scheme 1. Reagents and conditions: (a) β-glucosidase, allyl alcohol, H_2O, 37 °C; (b) pivaloyl chloride, pyridine, −15 °C; (c) **3**, $BF_3{\cdot}Et_2O$, MSAW 300, CH_2Cl_2, −78 °C; (d) $Pd[P(Ph)_3]_4$, CH_3COOH, 80 °C; (e) CCl_3CN, DBU, CH_2Cl_2, 0 °C.



Scheme 2. Reagents and conditions: (a) diosgenin, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (b) cholesterol, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (c) glycyrrhetic acid 30-methylester, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (d) ODS column chromatography (90% MeOH); (e) 3% KOH/MeOH, reflux; (f) 3% LiOH/MeOH, reflux.

of chacotriosides (e.g., compounds **13**–**18**) because the same synthesis procedure was not continually repeated.

In order to simplify the structure of the aglycone part and to avoid unnecessary glycosidic coupling reactions, double-chain neoglycolipids that mimicked glycosyl ceramides[16] were designed and synthesized. As shown in Scheme 3, fully protected chacotrioside **4** was deprotected with sodium hydroxide to yield allylchacotrioside **19** and ozonolyzed to yield an aldehyde. The aldehyde was used without further purification because it was unstable. It was treated with decylamine in the presence of sodium cyanoborohydride [17] to afford 1-octylaminoethyl-2-*O*-chacotrioside **20** (overall 73%). This single-chain chacotrioside **20** was selectively N-acylated with octanoyl chloride in a biphasic solution of tetrahydrofuran (THF)/2 M NaOAc[18] to afford *N*-octanoyl-1-octylaminoethyl-2-*O*-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside **22** in 97% yield; its reaction with tetradecyl chloride produced a double-chain chacotrioside **23** in 73% yield and was identical to the reaction of **20** with octanoyl chloride. Similarly, allylchacotrioside **19** was converted to a single-chain chacotrioside **21** (overall 71%), treated with tetradecylamine, and acylated with octanoyl or tetradecanoyl chloride to yield double-chain chacotriosides **24** (79%) and **25** (83%), respectively. This series of reactions, easy to carry out, appears suitable for the construction of a library of chacotriosyl lipids of varying lengths.



Scheme 3. Reagents and conditions: (a) 2 N NaOH/dioxane; (b) O_3, $(CH_3)_2S$, MeOH; (c) $NH_2(CH_2)_nCH_3$, $NaBH_3CN$, MeOH; (d) $CH_3(CH_2)_mCOCl$, 2 M $NaOCOCH_3$/THF.

Table 2. Cytotoxic activities of chacotriosyl derivatives

	GI_{50} (μM)	
	PC-12	HCT116
Cisplatin	0.33	2.85
Diosgenin	2.93	>12.1
13	>5.76	>5.76
14 (Dioscin)	2.29	3.17
15	>5.95	>5.95
16	>5.95	>5.95
17	>5.41	>5.41
18	>5.41	>5.41
20	>7.97	>7.97
21	>7.03	>7.03
22	>6.64	>6.64
23	>5.97	>5.97
24	>5.97	>5.97
25	1.10	>5.43
26 (Chacotriose)	>10.6	>10.6

Dioscin **14**, neosaponins (**13**, **15**–**18**), neoglycolipids (**20**–**25**), diosgenin, and chacotriose **26** were prepared from trisaccharide **5** by using alkaline hydrolysis; we examined the cytotoxicity of these compounds toward the PC-12 (human lung cancer)[19] and HCT116 (human colon cancer)[20] cell lines (Table 2). Dioscin **14** showed a moderate cytotoxicity despite having a lower activity than cisplatin. Since neosaponin **13**, which is an anomeric isomer of dioscin, did not show any activity, it was suggested that the naturally occurring β-form might be important. Because the other neosaponins (**15**–**18**) did not exhibit any activity, the spirostane-type aglycone part of **14** (diosgenin) could not be replaced by cholesterol and glycyrrhetic acid. Furthermore, both diosgenin and chacotriose **26**, which is a component of dioscin, did not show activity. Thus, these results suggest that the cytotoxicity of chacotriosyl derivatives depends on the β-glycosidic linkage of the chacotriosyl moiety and appropriate aglycone parts. Interestingly, similar to dioscin **14**, the double-chain chacotrioside **25** showed a moderate activity against PC-12 cell lines. Thus, a simplified conversion of the aglycone part to double-chain lipids instead of to diosgenin may provide a clue to understanding the mechanism by which glycoconjugates acquire cytotoxicity. Additionally, extending the lipid portion of the mimicked glycosyl ceramides is expected to improve their activity.

3. Experimental

3.1. General methods

Optical rotations were measured using the JASCO DIP-1000 KUY digital polarimeter ($l = 0.5$). ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL EX-270 MHz, JNM-GX 400 MHz, and JEOL α-500 MHz NMR spectrometers. Elucidation of the chemical structures was based on ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR experiments. Chemical shifts were reported in parts per million (ppm) relative to residual solvent peaks, and coupling constants (J) were reported in Hertz. FABMS and HRFABMS were obtained using a glycerol matrix in the positive ion mode by using a JEOL JMS-DX-303HF spectrometer. The HRESIMS was measured using a JMS-T100LC. Column chromatography was carried out on Silica Gel 60 (Kanto Chemical, Co, Inc., 70–230 mesh and 230–400 mesh); Amberlite IR-120B; MB-3 (Organo Co., Ltd); and chromatorex ODS (Fuji Silysia Chemical Co., Ltd) columns. TLC was performed on precoated Silica Gel 60 F_{254} plates (E. Merck).

3.2. Allyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (2)

Pivaloyl chloride (1.3 mL, 10.6 mmol) was added to a soln of **1** (910 mg, 4.1 mmol) in pyridine (4.0 mL) at −15 °C. After stirring for 2 h, the mixture was diluted with EtOAc. Next, the soln was washed with water and satd aq NaCl and concentrated under diminished pressure. The residue was purified by silica gel column chromatography (2:1 hexane–EtOAc) to yield **2** (1.1 g, 69%) as a colorless oil. $[\alpha]_D$ −5.80 (c 0.13, $CHCl_3$); HRFABMS: calcd for $C_{19}H_{33}O_8$ 389.2175, found m/z 389.2208 $[M+H]^+$; ^{1}H NMR (in $CDCl_3$): δ 5.86 (1H, m, H-2), 5.24 (1H, br d, J 17.2 Hz, H-3b), 5.15 (1H, br d, J 10.2 Hz, H-3a), 4.84 (1H, dd, J 9.6, 9.6 Hz, Glc H-3), 4.35 (1H, m, H-1b), 4.34 (1H, d, J 8.3 Hz, Glc H-1), 4.32 (1H, m, Glc H-6b), 4.22 (1H, m, Glc H-6a), 4.05 (1H, m, H-1a), 3.51 (1H, m, Glc H-5), 3.47 (1H, m, Glc H-2), 3.41 (1H, dd, J 9.6, 9.6 Hz, Glc H-4), 1.17, 1.15 (each 9H, s, $C(CH_3)_3 \times 2$).

3.3. Allyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)]-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (4)

$BF_3{\cdot}Et_2O$ (1.0 mL, 7.9 mmol) was added to a suspension of **4** (700 mg, 1.8 mmol) and molecular sieves AW 300 (4.0 g) in CH_2Cl_2 (5.0 mL) at −78 °C under nitrogen gas. After stirring for 1 h, rhamnosyl imidate **3** (2.4 g, 5.5 mmol) in CH_2Cl_2 (5.0 mL) was added to the mixture and stirred for 5 h at room temperature. The mixture was diluted with $CHCl_3$ and filtered using Celite. The filtrate was washed with satd aq $NaHCO_3$ and satd aq NaCl, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by silica gel column chromatography (3:1 hexane–EtOAc) to yield **4** (1.4 g, 84%) as a colorless oil. $[\alpha]_D$ +1.26 (c 0.13, $CHCl_3$); HRFABMS: calcd for $C_{43}H_{64}NaO_{22}$ 955.3787, found m/z 955.3859 $[M+Na]^+$; ^{1}H NMR (in $CDCl_3$): δ 5.91 (1H, m, H-2), 5.32–5.20 (3H, m, H-3, Glc H-3), 5.19–5.16 (2H, m, Rha H-3, Rha′ H-3), 5.16 (1H, m, Rha H-2), 5.05 (1H, m, Rha′ H-2), 5.02 (1H,

m, Rha H-4), 5.01 (1H, m, Rha′ H-4), 4.89 (1H, br s, Rha′ H-1), 4.82 (1H, br s, Rha H-1), 4.55 (1H, d, *J* 6.9 Hz, Glc H-1), 4.45 (1H, m, Glc H-6b), 4.35 (1H, m, H-1), 4.30 (1H, m, Glc H-5), 4.11 (1H, m, Rha′ H-5), 4.08 (1H, m, H-1a), 3.93 (1H, m, Rha H-5), 3.77 (2H, m, Glc H-4, 6a), 3.60 (1H, dd, *J* 7.6, 7.6 Hz, Glc H-2), 2.12, 2.11, 2.05, 2.04, 1.98, 1.96 (each 3H, s, $COCH_3 \times 6$), 1.23, 1.17 (each 9H, s, $C(CH_3)_3 \times 2$), 1.17 (6H, d, *J* 6.6 Hz, Rha H-6, Rha′ H-6); ^{13}C NMR (in $CDCl_3$): δ 133.4 (–CH–), 118.5 ($-CH_2$), 70.8 ($-OCH_2-$), 99.5, 77.7, 72.0, 77.4, 75.4, 62.7 (Glc C-1–6), 98.0, 69.9, 69.2, 70.5, 67.9, 17.1 (Rha [1→2] C-1–6), 97.3, 69.5, 68.7, 70.2, 66.7, 17.1 (Rha [1→4] C-1–6).

3.4. (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)]-3,6-di-*O*-pivaloyl-D-glucopyranose (5)

Fully protected trisaccharide **4** (200 mg, 0.21 mmol) and $Pd[P(Ph_3)]_4$ (150 mg, 0.13 mmol) were dissolved in acetic acid (3.0 mL) at 80 °C under nitrogen gas. After stirring for 1 h, the reaction solvent was removed, and the residue was purified by silica gel column chromatography (3:2 *n*-hexane–EtOAc) to yield **5** (150 mg, 78%) as a colorless oil. $[\alpha]_D$ +7.81 (*c* 0.13, $CHCl_3$); HRFABMS: calcd for $C_{40}H_{60}NaO_{22}$ 915.3474, found *m*/*z* 915.3580 $[M+H]^+$; ^1H NMR (in $CDCl_3$): δ 5.55 (1H, dd, *J* 9.8, 9.8 Hz, Glc H-3), 5.39 (1H, d, *J* 3.4 Hz, Glc H-1), 4.89 (1H, d, *J* 1.9 Hz, Rha′ H-1), 4.77 (1H, d, *J* 1.5 Hz, Rha H-1), 3.73 (1H, dd, *J* 9.8, 9.3 Hz, Glc H-4), 3.47 (1H, dd, *J* 9.8, 3.4 Hz, Glc H-2), 2.12×2, 2.04×2, 1.98, 1.96 (each 3H, s, $COCH_3 \times 6$), 1.23×2 (each 9H, s, $C(CH_3)_3 \times 2$), 1.22 (6H, d, *J* 6.3 Hz, Rha H-6, Rha′ H-6); ^{13}C NMR (in $CDCl_3$): δ 91.8, 80.2, 70.7, 77.2, 70.8, 61.9 (Glc C-1–6), 99.9, 69.6, 68.7, 70.6, 67.9, 17.4 (Rha [1→2] C-1–6), 97.5, 68.9, 68.7, 70.1, 67.2, 17.2 (Rha [1→4] C-1–6).

3.5. (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-3,6-di-*O*-pivaloyl-D-α-glucopyranosyl trichloroacetimidate (6)

Trichloroacetonitrile (0.40 mL, 3.9 mmol) was added to a soln of trisaccharide **5** (150 mg, 0.17 mmol) in CH_2Cl_2 (3.0 mL) at 0 °C under nitrogen gas. After stirring for 30 min, DBU (0.050 mL, 0.32 mmol) was added to the mixture and stirred for 1 h. The mixture was concentrated under diminished pressure, and the residue was purified by silica gel column chromatography (2:1 hexane–EtOAc) to yield **6** (150 mg, 86%) as a colorless oil. ^1H NMR (in $CDCl_3$): δ 8.77 (1H, s, NH), 6.42 (1H, d, *J* 3.7 Hz, Glc H-1), 5.64 (1H, dd, *J* 9.8, 9.1 Hz, Glc H-3), 4.91 (1H, d, *J* 1.8 Hz, Rha′ H-1), 4.81 (1H, s, Rha H-1), 2.12, 2.04×2, 1.98, 1.97, 1.94 (each 3H, s, $COCH_3 \times 6$), 1.23, 1.21 (each 9H, s, $C(CH_3)_3 \times 2$), 1.16 (3H, d, *J* 6.6 Hz, Rha H-6), 1.16 (3H, d, *J* 6.1 Hz, Rha′ H-6).

3.6. General procedure for the preparation of compounds 7–12

Chacotriosyl imidate **6** (0.05 mmol), a glycosyl acceptor (1.0 mmol), and molecular sieves AW 300 (800 mg) were suspended in CH_2Cl_2 (8.0 mL) at 20 °C. After stirring for 30 min, $BF_3{\cdot}Et_2O$ (10% soln in CH_2Cl_2, 2.0 μmol) was added dropwise to the mixture. The mixture was stirred for 2 h at room temperature, diluted with $CHCl_3$, and filtered using Celite. The filtrate was washed with satd aq $NaHCO_3$ and satd aq NaCl, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by column chromatography to yield an anomeric mixture of protected glycosides. The mixture was separated by ODS column chromatography to give α-glycoside and β-glycoside, respectively.

3.6.1. Diosgenin-3β-yl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)]-3,6-di-*O*-pivaloyl-D-glucopyranoside (7, 8). Glycosylation of **6** (48 mg, 0.046 mmol) with diosgenin (400 mg, 0.97 mmol) was accomplished as described in Section 3.6, and purified by column chromatography (2:1 hexane–EtOAc) to yield a mixture of **7** and **8** as a colorless oil. The mixture was separated by ODS column chromatography (9:1 MeOH–water) to give both **7** (26 mg, 44%) and **8** (12 mg, 20%) as white solids. Data for α-glycoside (**7**): $[\alpha]_D$ −2.9 (*c* 0.24, $CHCl_3$); HRFABMS: calcd for $C_{67}H_{100}NaO_{24}$ 1311.6500, found *m*/*z* 1311.6210 $[M+Na]^+$; ^1H NMR (in $CDCl_3$): δ 4.96 (1H, d, *J* 3.7 Hz, Glc H-1), 4.81 (1H, s, Rha H-1), 4.64 (1H, s, Rha′ H-1), 2.03, 2.02, 1.95, 1.93, 1.89, 1.86 (each 3H, s, $COCH_3 \times 6$), 1.14, 1.11 (each 9H, s, $C(CH_3)_3 \times 2$), 1.09 (3H, d, *J* 5.7 Hz, Rha H-6), 1.08 (3H, d, *J* 6.7 Hz, Rha′ H-6), 0.98 (3H, s, H-19), 0.88 (3H, d, *J* 7.3 Hz, H-21), 0.79 (3H, s, H-18), 0.70 (3H, d, *J* 6.1 Hz, H-27); ^{13}C NMR (in $CDCl_3$): δ 37.1, 28.8, 79.1, 40.1, 140.1, 122.2, 32.0, 31.4, 50.1, 36.8, 20.8, 39.8, 40.2, 56.5, 32.1, 80.8, 62.1, 16.3, 19.4, 41.6, 14.5, 109.3, 31.8, 28.0, 30.3, 66.8, 17.1 (C-1–27), 97.5, 80.0, 71.0, 77.4, 70.9, 62.7 (Glc C-1–6), 99.8, 70.2, 68.8, 71.0, 67.8, 17.6 (Rha [1→2] C-1–6), 96.2, 69.6, 68.0, 70.9, 66.8, 17.2 (Rha [1→4] C-1–6). Data for β-glycoside (**8**): $[\alpha]_D$ −32.7 (*c* 0.19, $CHCl_3$); HRFABMS: calcd for $C_{67}H_{100}NaO_{24}$ 1311.6500, found *m*/*z* 1311.6690 $[M+Na]^+$; ^1H NMR (in $CDCl_3$): δ 4.77 (1H, s, Rha H-1), 4.70 (1H, s, Rha′ H-1), 4.47 (1H, d, *J* 7.3 Hz, Glc H-1), 1.99, 1.97, 1.91, 1.89, 1.84, 1.82 (each 3H, s, $COCH_3 \times 6$), 1.09, 1.04 (each 9H, s, $C(CH_3)_3 \times 2$), 1.06 (3H, d, *J* 6.1 Hz, Rha H-6), 1.03 (3H, d, *J* 6.1 Hz, Rha′ H-6), 0.88 (3H, s, H-19), 0.85 (3H, d, *J* 6.7 Hz, H-21), 0.67 (3H, d, *J* 6.1 Hz, H-27), 0.66 (3H, s, H-18); ^{13}C

NMR (in $CDCl_3$): δ 37.4, 29.0, 79.1, 38.5, 140.3, 122.3, 32.1, 31.7, 50.3, 37.1, 20.9, 40.0, 40.5, 56.7, 32.3, 81.0, 62.4, 16.5, 19.5, 41.9, 14.7, 109.5, 31.6, 27.4, 30.5, 67.0, 17.4 (C-1–27), 99.1, 76.4, 75.4, 77.2, 72.5, 63.3 (Glc C-1–6), 97.6, 70.2, 69.3, 71.3, 68.3, 17.5 (Rha [1→2] C-1–6), 97.3, 69.7, 69.0, 70.8, 66.7, 17.4 (Rha [1→4] C-1–6).

3.6.2. Cholesterol-3β-yl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)]-3,6-di-*O*-pivaloyl-D-glucopyranoside (9, 10). Glycosylation of **6** (50 mg, 0.048 mmol) with cholesterol (180 mg, 0.47 mmol) was accomplished as described in Section 3.6, and purified by column chromatography (2:1 hexane–EtOAc) to yield a mixture of **9** and **10** as a colorless oil. The mixture was separated by ODS column chromatography (9:1 MeOH–water) to give both **9** (27 mg, 45%) and **10** (20 mg, 32%) as white solids. Data for α-glycoside (**9**): $[\alpha]_D$ +33.1 (*c* 0.18, $CHCl_3$); HRFABMS: calcd for $C_{67}H_{104}NaO_{22}$ 1283.6920, found *m*/*z* 1283.6700 $[M+Na]^+$; ^1H NMR ($CDCl_3$): δ 5.05 (1H, d, *J* 3.7 Hz, Glc H-1), 4.90 (1H, s, Rha H-1), 4.73 (1H, s, Rha′ H-1), 2.12, 2.11, 2.04, 2.02, 1.98, 1.95 (each 3H, s, $COCH_3 \times 6$), 1.22, 1.20 (each 9H, s, $C(CH_3)_3 \times 2$), 1.18 (3H, d, *J* 6.7 Hz, Rha H-6), 1.17 (3H, d, *J* 6.7 Hz, Rha′ H-6), 1.05 (3H, s, H-19), 0.92 (3H, d, *J* 6.1 Hz, H-21), 0.87 (3H, d, *J* 6.7 Hz, H-27), 0.86 (3H, d, *J* 6.7 Hz, H-26), 0.69 (3H, s, H-18); ^{13}C NMR (in $CDCl_3$): δ 37.2, 28.2, 79.2, 40.1, 140.4, 122.5, 31.9, 31.9, 50.2, 36.6, 21.1, 28.0, 42.3, 56.8, 24.3, 39.8, 56.2, 11.9, 19.4, 35.8, 18.7, 36.2, 23.8, 39.5, 20.8, 22.8, 22.6 (C-1–27), 97.5, 80.0, 71.1, 77.5, 70.9, 62.7 (Glc C-1–6), 99.9, 69.6, 68.8, 71.1, 67.8, 17.6 (Rha [1→2] C-1–6), 96.2, 69.8, 68.0, 70.2, 66.9, 17.2 (Rha [1→4] C-1–6). Data for β-glycoside (**10**): $[\alpha]_D$ −33.3 (*c* 0.16, $CHCl_3$); HRFABMS: calcd for $C_{67}H_{104}NaO_{22}$ 1283.6920, found *m*/*z* 1283.6800 $[M+Na]^+$; ^1H NMR (in $CDCl_3$): δ 4.89 (1H, s, Rha H-1), 4.83 (1H, s, Rha′ H-1), 4.60 (1H, d, *J* 7.3 Hz, Glc H-1), 2.12, 2.10, 2.04, 2.01, 1.97, 1.95 (each 3H, s, $COCH_3 \times 6$), 1.21, 1.17 (each 9H, s, $C(CH_3)_3 \times 2$), 1.11 (3H, d, *J* 6.7 Hz, Rha H-6), 1.10 (3H, d, *J* 6.7 Hz, Rha′ H-6), 0.99 (3H, s, H-19), 0.92 (3H, d, *J* 6.7 Hz, H-21), 0.87 (3H, d, *J* 6.7 Hz, H-27), 0.86 (3H, d, *J* 6.7 Hz, H-26), 0.67 (3H, s, H-18); ^{13}C NMR (in $CDCl_3$): δ 37.4, 28.2, 78.8, 39.8, 139.9, 122.4, 31.9, 31.9, 50.2, 36.7, 21.1, 28.2, 42.3, 56.8, 24.3, 39.5, 56.2, 11.9, 19.2, 35.8, 18.7, 36.2, 23.8, 39.6, 20.8, 22.8, 22.6 (C-1–27), 98.8, 76.2, 75.1, 78.8, 72.2, 63.1 (Glc C-1–6), 97.4, 69.9, 69.1, 71.1, 68.0, 17.3 (Rha [1→2] C-1–6), 97.1, 69.5, 68.7, 70.6, 66.5, 17.2 (Rha [1→4] C-1–6).

3.6.3. 30-Methyl glycyrrhetinate-3β-yl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→4)-[(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)]-3,6-di-*O*-pivaloyl-D-glucopyranoside (11, 12). Glycosylation of **6** (130 mg, 0.13 mmol) with 30-methyl glycyrrhetinate (870 mg, 1.7 mmol) was accomplished as described in Section 3.6, and purified by column chromatography (2:1:1 hexane–EtOAc–$CHCl_3$) to yield a mixture of **11** and **12** as a colorless oil. The mixture was separated by ODS column chromatography (9:1 MeOH–water) to give both **11** (71 mg, 41%) and **12** (39 mg, 23%) as white solids. Data for α-glycoside (**11**): $[\alpha]_D$ +73.1 (*c* 0.26, $CHCl_3$); HRFABMS: calcd for $C_{71}H_{106}NaO_{25}$ 1381.6920, found *m*/*z* 1381.6860 $[M+Na]^+$; ^1H NMR (in $CDCl_3$): δ 5.68 (1H, s, H-12), 5.00 (1H, d, *J* 3.7 Hz, Glc H-1), 4.92 (1H, s, Rha H-1), 4.74 (1H, s, Rha′ H-1), 3.69 (3H, s, $COOCH_3$), 2.34 (1H, s, H-9), 2.12×2, 2.04, 2.01, 1.97, 1.94 (each 3H, s, $COCH_3 \times 6$), 1.24, 1.20 (each 9H, s, $C(CH_3)_3 \times 2$), 1.16×2, 1.15, 1.13×2, 0.97, 0.81 (each 3H, s, *tert*-$CH_3 \times 7$); ^{13}C NMR (in $CDCl_3$): δ 38.6, 26.5, 91.2, 39.1, 55.4, 17.4, 32.8, 44.1, 61.8, 37.0, 200.2, 128.5, 169.3, 45.5, 26.5, 26.5, 31.9, 48.4, 41.1, 43.2, 31.2, 37.8, 28.3, 16.4, 16.4, 18.7, 23.3, 28.7, 28.5, 176.9 (C-1–30), 51.8 ($COOCH_3$), 96.1, 79.6, 71.2, 78.9, 70.1, 63.1 (Glc C-1–6), 99.5, 69.5, 68.7, 71.2, 67.9, 17.6 (Rha [1→2] C-1–6), 97.1, 68.8, 68.1, 70.6, 66.8, 17.2 (Rha [1→4] C-1–6). Data for β-glycoside (**12**): $[\alpha]_D$ +26.4 (*c* 0.21, $CHCl_3$); HRFABMS: calcd for $C_{71}H_{106}NaO_{25}$ 1381.6920, found *m*/*z* 1381.6940 $[M+Na]^+$; ^1H NMR (in $CDCl_3$): δ 5.68 (1H, s, H-12), 4.92 (1H, s, Rha H-1), 4.89 (1H, s, Rha′ H-1), 4.68 (1H, d, *J* 6.1 Hz, Glc H-1), 3.69 (3H, s, $COOCH_3$), 2.33 (1H, s, H-9), 2.13, 2.11, 2.04, 2.02, 1.97, 1.95 (each 3H, s, $COCH_3 \times 2$), 1.22, 1.19 (each 9H, s, $C(CH_3)_3$), 1.16, 1.15×2, 1.13, 1.12, 0.84, 0.81 (each 3H, s, *tert*-$CH_3 \times 7$); ^{13}C NMR (in $CDCl_3$): δ 39.4, 26.5, 89.2, 39.4, 55.5, 17.4, 32.8, 44.1, 61.8, 36.9, 200.1, 128.6, 169.1, 45.5, 26.5, 26.5, 31.9, 48.4, 41.1, 43.2, 31.2, 37.8, 28.1, 16.5, 16.5, 18.7, 23.4, 28.5, 28.4, 176.9 (C-1–30), 51.8 ($COOCH_3$), 102.2, 76.7, 75.0, 77.2, 72.4, 63.4 (Glc C-1–6), 97.3, 69.9, 68.9, 71.1, 67.9, 17.4 (Rha [1→2] C-1–6), 96.0, 69.5, 68.8, 70.6, 66.6, 17.2 (Rha [1→4] C-1–6).

3.7. General procedure for the preparation of compounds 13–18

The protected neoglycoside (0.1 mmol) was dissolved in 3% KOH/MeOH (5.0 mL) and heated at 65 °C. After stirring for 20 h, the mixture was purified by column chromatography (MCI gel, $CHP_{20}P$; water, 250 mL; MeOH, 150 mL), and the organic solvent was concentrated under diminished pressure to afford a neosaponin.

3.7.1. Diosgenin-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-D-glucopyranoside (13). Compound **7** (102 mg, 0.079 mmol) was deprotected as described in Section 3.7 to give **13** (67 mg, 98%) as a white solid: $[\alpha]_D$ −26.1 (*c* 0.11, MeOH); HRESIMS: calcd for $C_{45}H_{72}NaO_{16}$ 891.4718, found *m*/*z* 891.4733 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 5.95 (1H, s, Rha H-1), 5.86 (1H, s, Rha′ H-1), 5.52 (1H, d, *J* 3.7 Hz,

Glc H-1), 1.70 (3H, d, *J* 6.1 Hz, Rha H-6), 1.68 (3H, d, *J* 6.1 Hz, Rha′ H-6), 1.15 (3H, d, *J* 6.7 Hz, H-21), 0.88 (3H, s, H-19), 0.84 (3H, s, H-18), 0.70 (3H, d, *J* 5.5 Hz, H-27); ^{13}C NMR (in C_5D_5N): δ 37.3, 29.3, 79.6, 40.8, 141.1, 121.6, 32.2, 31.6, 50.2, 37.0, 21.1, 39.9, 40.5, 56.7, 32.3, 81.1, 62.9, 16.4, 19.3, 42.0, 15.0, 109.3, 31.8, 28.7, 30.6, 66.9, 17.3 (C-1–27), 98.2, 79.4, 72.8, 78.7, 72.3, 61.5 (Glc C-1–6), 104.2, 72.8, 72.8, 74.0, 70.5, 18.7 (Rha [1→2] C-1–6), 103.0, 72.6, 72.8, 74.0, 70.3, 18.5 (Rha [1→4] C-1–6).

3.7.2. Diosgenin-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (14).[21] Compound **8** (41 mg, 0.032 mmol) was deprotected as described in Section 3.7 to give **14** (27 mg, 97%) as a white solid: $[\alpha]_D$ −102.0 (*c* 0.11, MeOH); HRESIMS: calcd for $C_{45}H_{72}NaO_{16}$ 891.4718, found *m*/*z* 891.4785 $[M+Na]^+$; ^{1}H NMR (in C_5D_5N): δ 6.39 (1H, s, Rha H-1), 5.85 (1H, s, Rha′ H-1), 5.52 (1H, d, *J* 7.3 Hz, Glc H-1), 1.77 (3H, d, *J* 6.1 Hz, Rha H-6), 1.63 (3H, d, *J* 6.1 Hz, Rha′ H-6), 1.14 (3H, d, *J* 7.3 Hz, H-21), 1.06 (3H, s, H-19), 0.84 (3H, s, H-18), 0.70 (3H, d, *J* 5.5 Hz, H-27).

3.7.3. Cholesterol-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-D-glucopyranoside (15). Compound **9** (40 mg, 0.032 mmol) was deprotected as described in Section 3.7 to give **15** (27 mg, 99%) as a white solid: $[\alpha]_D$ +5.49 (*c* 0.11, MeOH); HRESIMS: calcd for $C_{45}H_{76}NaO_{14}$ 863.5132, found *m*/*z* 863.5180 $[M+Na]^+$; ^{1}H NMR (in C_5D_5N): δ 5.97 (1H, s, Rha H-1), 5.87 (1H, s, Rha′ H-1), 5.53 (1H, d, *J* 3.7 Hz, Glc H-1), 1.71 (3H, d, *J* 6.7 Hz, Rha H-6), 1.69 (3H, d, *J* 6.7 Hz, Rha′ H-6), 0.98 (3H, d, *J* 6.1 Hz, H-21), 0.91 (3H, s, H-19), 0.90 (3H, d, *J* 6.7 Hz, H-27), 0.89 (3H, d, *J* 6.7 Hz, H-26), 0.66 (3H, s, H-18); ^{13}C NMR (in C_5D_5N): δ 37.4, 28.7, 79.6, 40.9, 141.2, 121.0, 32.2, 32.1, 50.4, 36.9, 21.3, 28.5, 42.5, 56.9, 24.5, 40.0, 56.4, 12.0, 19.4, 36.1, 18.9, 36.5, 24.2, 39.8, 20.8, 23.0, 22.7 (C-1–27), 98.3, 79.4, 72.8, 78.7, 72.3, 61.5 (Glc C-1–6), 104.2, 72.8, 72.9, 74.0, 70.5, 18.7 (Rha [1→2] C-1–6), 103.2, 72.6, 72.8, 74.0, 70.4, 18.6 (Rha [1→4] C-1–6).

3.7.4. Cholesterol-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (16). Compound **10** (30 mg, 0.024 mmol) was deprotected as described in Section 3.7 to give **16** (22 mg, 98%) as a white solid: $[\alpha]_D$ −42.2 (*c* 0.10, MeOH); HRESIMS: calcd for $C_{45}H_{76}NaO_{14}$ 863.5132, found *m*/*z* 863.5165 $[M+Na]^+$; ^{1}H NMR (in C_5D_5N): δ 6.78 (1H, d, *J* 6.1 Hz, Glc H-1), 6.40 (1H, s, Rha H-1), 5.86 (1H, s, Rha′ H-1), 1.78 (3H, d, *J* 6.1 Hz, Rha H-6), 1.64 (3H, d, *J* 6.1 Hz, Rha′ H-6), 1.08 (3H, s, H-19), 0.97 (3H, d, *J* 6.7 Hz, H-21), 0.90 (3H, d, *J* 6.7 Hz, H-27), 0.89 (3H, d, *J* 6.7 Hz, H-26), 0.66 (3H, s, H-18); ^{13}C NMR (in C_5D_5N): δ 37.6, 30.2, 78.7, 39.0, 141.0, 122.0, 32.3, 32.1, 50.5, 37.0, 21.3, 28.5, 42.5, 56.9, 24.6, 40.0, 56.4, 12.0, 19.5, 36.1, 18.9, 36.5, 24.2, 39.8, 20.8, 23.0, 22.7 (C-1–27), 100.3, 78.2, 76.9, 78.0, 77.8, 61.3 (Glc C-1–6), 102.2, 72.6, 72.7, 74.2, 69.5, 18.7 (Rha [1→2] C-1–6), 102.9, 72.6, 72.9, 73.9, 70.4, 18.5 (Rha [1→4] C-1–6).

3.7.5. Glycyrrhetic acid-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-D-glucopyranoside (17). Compound **11** (60 mg, 0.044 mmol) was deprotected as described in Section 3.7 to give **17** (39 mg, 97%) as a white solid: $[\alpha]_D$ +56.5 (*c* 0.13, MeOH); HRESIMS: calcd for $C_{48}H_{76}NaO_{17}$ 947.4980, found *m*/*z* 947.5025 $[M+Na]^+$; ^{1}H NMR (in C_5D_5N): δ 5.85 (1H, s, H-12), 5.49 (1H, d, *J* 3.7 Hz, Glc H-1), 4.79 (1H, s, Rha H-1), 4.70 (1H, s, Rha′ H-1), 2.33 (1H, s, H-9), 1.31, 1.30, 1.24, 1.13, 1.08, 0.98, 0.71 (each 3H, s, *tert*-CH_3 × 7); ^{13}C NMR (in C_5D_5N): δ 38.9, 26.4, 83.2, 42.6, 55.1, 17.4, 32.6, 44.0, 61.7, 37.0, 199.2, 128.1, 169.3, 45.2, 26.5, 26.4, 31.8, 48.5, 41.9, 43.2, 31.6, 38.2, 28.3, 16.3, 16.7, 18.5, 23.1, 28.7, 28.4, 181.3 (C-1–30), 95.5, 79.4, 72.3, 78.7, 71.7, 61.2 (Glc C-1–6), 102.6, 72.5, 72.7, 73.4, 70.1, 18.5 (Rha [1→2] C-1–6), 103.1, 72.0, 72.7, 73.4, 69.6, 18.5 (Rha [1→4] C-1–6).

3.7.6. Glycyrrhetic acid-3β-yl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (18). Compound **12** (38 mg, 0.028 mmol) was deprotected as described in Section 3.7 to give **18** (25 mg, 96%) as a white solid: $[\alpha]_D$ +27.8 (*c* 0.10, MeOH); HRESIMS: calcd for $C_{48}H_{76}NaO_{17}$ 947.4980, found *m*/*z* 947.5123 $[M+Na]^+$; ^{1}H NMR (in C_5D_5N): δ 5.86 (1H, s, H-12), 5.49 (1H, d, *J* 7.3 Hz, Glc H-1), 4.86 (1H, s, Rha H-1), 4.70 (1H, s, Rha′ H-1), 2.47 (1H, s, H-9), 1.73 (3H, d, *J* 6.1 Hz, Rha H-6), 1.59 (3H, d, *J* 6.1 Hz, Rha′ H-6), 1.30, 1.28, 1.27, 1.25, 1.23, 1.09, 0.74 (each 3H, s, *tert*-CH_3 × 7); ^{13}C NMR (in C_5D_5N): δ 39.5, 26.5, 88.4, 39.5, 55.3, 17.3, 32.6, 43.9, 61.7, 37.0, 199.1, 128.2, 169.3, 45.2, 26.5, 26.3, 31.7, 48.4, 41.9, 43.2, 31.5, 38.2, 28.3, 16.3, 16.7, 18.5, 23.2, 28.6, 28.3, 181.2 (C-1–30), 101.2, 79.1, 76.3, 77.8, 77.6, 61.2 (Glc C-1–6), 102.6, 71.9, 72.1, 73.7, 69.1, 18.5 (Rha [1→2] C-1–6), 104.1, 72.0, 72.3, 73.4, 70.0, 18.4 (Rha [1→4] C-1–6).

3.8. Allyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (19)

Fully protected trisaccharide **4** (500 mg, 0.54 mmol) was dissolved in 2 M NaOH–dioxane (1:1, 4.0 mL) at room temperature. After stirring for 14 h, the mixture was subjected to Amberlite IR-120B (H^+ form), and the products were extracted using MeOH and concentrated under diminished pressure to yield **19** (280 mg) as a colorless oil. $[\alpha]_D$ +20.1 (*c* 0.10, MeOH); FABMS (*m*/*z*): 513 $[M+H]^+$; ^{1}H NMR (in C_5D_5N): δ 6.33 (1H, s, Rha H-1), 6.07 (1H, m, H-2), 5.82 (1H, s, Rha′ H-1),

5.37 (1H, br d, *J* 17.7 Hz, H-3a), 5.15 (1H, d, *J* 10.4 Hz, H-3b), 4.42 (1H, d, *J* 7.3 Hz, Glc H-1), 4.34 (1H, m, H-1a), 4.05 (1H, m, H-1b), 1.69, 1.61 (each 3H, d, *J* 6.1 Hz, Rha H-6, Rha′ H-6).

3.9. General procedure for the preparation of compounds 20 and 21

O_3 gas was added to a soln of allyl chacotrioside **19** (0.4 mmol) in MeOH (7.0 mL) at a temperature below −70 °C. After the color of the mixture changed to blue, O_3 gas was substituted with nitrogen gas, and dimethyl sulfide (10.0 mmol) was added to the mixture. The mixture was stirred for 18 h and concentrated under diminished pressure to yield an aldehyde. The aldehyde (0.4 mmol) was dissolved in MeOH (3.0 mL) containing alkyl amine (2.0 mmol) adjusted to pH 6–7, and $NaBH_3CN$ (0.5 mmol) was added. After stirring for 40 h, the mixture was concentrated under diminished pressure, and the residue was purified by silica gel column chromatography.

3.9.1. Octylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (20). This was synthesized as described in Section 3.9 using compound **19** (200 mg, 0.39 mmol) and octylamine (0.3 mL, 2.3 mmol). The product was purified by column chromatography (6:4:0.8:0.2 $CHCl_3$–MeOH–H_2O–NH_4OH) to give compound **20** (180 mg, 73%) as a colorless oil: $[\alpha]_D$ +27.8 (*c* 0.10, MeOH); HRFABMS: calcd for $C_{28}H_{54}NO_{14}$ 628.3544, found *m*/*z* 628.3438 $[M+H]^+$; ^1H NMR (in C_5D_5N): δ 6.11 (1H, s, Rha H-1), 5.66 (1H, s, Rha′ H-1), 4.73 (1H, d, *J* 7.3 Hz, Glc H-1), 1.25–1.11 (12H, m, $-CH_2-\times 6$), 0.81 (3H, t, *J* 7.3 Hz, $-CH_3$); ^{13}C NMR (in C_5D_5N): δ 65.5 ($-OCH_2CH_2NH-$), 48.3 ($-OCH_2CH_2NH-$), 48.1 ($-NHCH_2C_7H_{15}$), 31.9, 29.3 × 2, 27.1, 26.3, 22.8 ($-CH_2-\times 6$), 14.2 ($-CH_3$), 102.2, 78.8, 77.0, 78.1, 77.1, 61.3 (Glc C-1–6), 102.6, 72.6, 72.4, 74.1, 70.5, 18.7 (Rha [1→2] C-1–6), 102.9, 72.6, 72.2, 73.8, 69.8, 18.5 (Rha [1→4] C-1–6).

3.9.2. Tetradecylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (21). This was synthesized as described in Section 3.9 using compound **19** (100 mg, 0.20 mmol) and tetradecylamine (150 mg, 0.70 mmol). The product was purified by column chromatography (7:3:0.4:0.1 $CHCl_3$–MeOH–H_2O–NH_4OH) to give compound **21** (98 mg, 71%) as a colorless oil: $[\alpha]_D$ +27.8 (*c* 0.10, MeOH); HRFABMS: calcd for $C_{34}H_{66}NO_{14}$ 712.4483, found *m*/*z* 712.4595 $[M+H]^+$; ^1H NMR (in C_5D_5N): δ 6.12 (1H, s, Rha H-1), 5.67 (1H, s, Rha′ H-1), 4.73 (1H, d, *J* 7.6 Hz, Glc H-1), 1.28–1.17 (24H, m, $-CH_2-\times 12$), 0.87 (3H, t, *J* 6.7 Hz, $-CH_3$); ^{13}C NMR (in C_5D_5N): δ 65.4 ($-OCH_2CH_2NH-$), 48.3 ($-OCH_2-CH_2NH-$), 48.1 ($-NHCH_2C_{13}H_{27}$), 32.1, 30.0 × 2, 29.9 × 2 29.8, 29.7, 29.6, 29.4, 27.2, 26.3, 23.0 ($-CH_2-\times 12$), 14.2 ($-CH_3$), 102.2, 78.8, 77.0, 78.2, 77.1, 61.3 (Glc C-1–6), 102.6, 72.6, 72.4, 74.1, 70.5, 18.7 (Rha [1→2] C-1–6), 102.9, 72.6, 72.2, 73.8, 69.8, 18.5 (Rha [1→4] C-1–6).

3.10. General procedure for the preparation of compounds 22–25

Carboxylic acid chloride (0.3 mmol) was added to a soln of chacotriosyl derivative (0.1 mmol) in 2 M NaOAc/THF (2:3, 5.0 mL) at room temperature. After stirring for 2 h, the mixture was concentrated under diminished pressure, and the residue was purified by silica gel column chromatography.

3.10.1. *N*-Octanoyl-octylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (22). This was synthesized as described in Section 3.10 using compound **20** (60 mg, 0.10 mmol) and octanoyl chloride (0.04 mL, 0.25 mmol). The product was purified by column chromatography (7:3:0.3:0.2 $CHCl_3$–MeOH–H_2O–NH_4OH) to give compound **22** (70 mg, 97%) as a colorless oil: $[\alpha]_D$ +67.1 (*c* 0.10, MeOH); HRESIMS: calcd for $C_{36}H_{67}NNaO_{15}$ 776.4408, found *m*/*z* 776.4398 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 6.29 (1H, s, Rha H-1), 5.78 (1H, s, Rha′ H-1), 4.76 (1H, d, *J* 7.3 Hz, Glc H-1), 1.36–1.22 (22H, m, $-CH_2-\times 11$), 0.83 (6H, t, *J* 6.6 Hz, $-CH_3\times 2$); ^{13}C NMR (in C_5D_5N): δ 67.8 ($-OCH_2CH_2NH-$), 49.1 ($-OCH_2-CH_2NH-$), 47.8 ($-NHCH_2C_7H_{15}$), 172.8 ($-COCH_2-$), 46.1 ($-COCH_2-$), 22.9–33.5 ($-CH_2-\times 11$), 14.3 × 2 ($-CH_3\times 2$), 102.0, 78.6, 77.5, 78.0, 77.0, 61.2 (Glc C-1–6), 102.9, 72.8, 72.4, 74.1, 70.4, 18.7 (Rha [1→2] C-1–6), 102.9, 72.7, 72.3, 73.8, 69.8, 18.4 (Rha [1→4] C-1–6).

3.10.2. *N*-Tetradecanoyl-octylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (23). This was synthesized as described in Section 3.10 using compound **20** (57 mg, 0.09 mmol) and tetradecanoyl chloride (0.05 mL, 0.18 mmol). The product was purified by column chromatography (8:2:0.2 $CHCl_3$–MeOH–NH_4OH) to give compound **23** (56 mg, 73%) as a colorless oil: $[\alpha]_D$ +18.2 (*c* 0.10, MeOH); HRESIMS: calcd for $C_{42}H_{79}NNaO_{15}$ 860.5347, found *m*/*z* 860.5364 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 6.28 (1H, s, Rha H-1), 5.76 (1H, s, Rha′ H-1), 4.74 (1H, d, *J* 6.7 Hz, Glc H-1), 1.39–1.24 (34H, m, $-CH_2-\times 17$), 0.84 (6H, t, *J* 6.7 Hz, $-CH_3\times 2$); ^{13}C NMR (in C_5D_5N): δ 67.8 ($-OCH_2CH_2NH-$), 49.1 ($-OCH_2-CH_2NH-$), 47.8 ($-NHCH_2C_7H_{15}$), 172.8 ($-COCH_2-$), 46.2 ($-COCH_2-$), 22.9–33.5 ($-CH_2-\times 17$), 14.3 × 2 ($-CH_3\times 2$), 102.8, 78.6, 77.5, 78.0, 77.0, 61.2 (Glc C-1–6), 102.9, 72.8, 72.5, 74.1, 70.4, 18.7 (Rha [1→2] C-1–6), 102.9, 72.7, 72.4, 73.9, 69.7, 18.5 (Rha [1→4] C-1–6).

3.10.3. *N*-Octanoyl-tetradecylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (24). This was synthesized as described in Section 3.10 using compound **21** (80 mg, 0.11 mmol) and octanoyl chloride (0.06 mL, 0.37 mmol). The product was purified by column chromatography (4:1:0.1 $CHCl_3$–MeOH–NH_4OH) to give compound **24** (74 mg, 79%) as a colorless oil: $[\alpha]_D$ +11.8 (*c* 0.07, MeOH); HRESIMS: calcd for $C_{42}H_{79}NNaO_{15}$ 860.5347, found *m*/*z* 860.5336 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 6.32 (1H, s, Rha H-1), 5.80 (1H, s, Rha′ H-1), 4.77 (1H, d, *J* 6.7 Hz, Glc H-1), 1.36–1.18 (34H, m, $-CH_2-\times 17$), 0.85 (6H, t, *J* 6.7 Hz, $-CH_3\times 2$); ^{13}C NMR (in C_5D_5N): δ 68.0 ($-OCH_2CH_2NH-$), 49.1 ($-OCH_2-CH_2NH-$), 47.8 ($-NHCH_2C_{13}H_{27}$), 176.0 ($-COCH_2-$), 46.3 ($-COCH_2-$), 23.0–33.5 ($-CH_2-\times 17$), 14.3 × 2 ($-CH_3\times 2$), 102.0, 78.7, 77.5, 78.0, 77.0, 61.3 (Glc C-1–6), 103.0, 72.8, 72.5, 74.1, 70.5, 18.8 (Rha [1→2] C-1–6), 103.0, 72.7, 72.4, 73.9, 69.8, 18.5 (Rha [1→4] C-1–6).

3.10.4. *N*-Tetradecanoyl-tetradecylaminoethyl α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (25). This was synthesized as described in Section 3.10 using compound **21** (80 mg, 0.11 mmol) and tetradecanoyl chloride (0.09 mL, 0.37 mmol). The product was purified by column chromatography (9:1:0.1 $CHCl_3$–MeOH–NH_4OH) to give compound **25** (85 mg, 83%) as a colorless oil: $[\alpha]_D$ +33.4 (*c* 0.10, MeOH); HRESIMS: calcd for $C_{48}H_{91}NNaO_{15}$ 944.6286, found *m*/*z* 944.6263 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 6.30 (1H, s, Rha H-1), 5.80 (1H, s, Rha′ H-1), 4.78 (1H, d, *J* 6.7 Hz, Glc H-1), 1.39–1.25 (46H, m, $-CH_2-\times 23$), 0.86 (6H, t, *J* 7.1 Hz, $-CH_3\times 2$); ^{13}C NMR (in C_5D_5N): δ 67.8 ($-OCH_2CH_2NH-$), 49.1 ($-OCH_2CH_2NH-$), 47.8 ($-NHCH_2C_{13}H_{27}$), 172.8 ($-COCH_2-$), 46.1 ($-COCH_2-$), 22.8–33.5 ($-CH_2-\times 23$), 14.3 × 2 ($-CH_3\times 2$), 102.0, 78.6, 77.5, 78.0, 77.1, 61.2 (Glc C-1–6), 102.9, 72.8, 72.5, 74.1, 70.5, 18.8 (Rha [1→2] C-1–6), 103.0, 72.7, 72.4, 73.9, 69.7, 18.5 (Rha [1→4] C-1–6).

3.11. α-L-Rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-D-glucopyranose (26)

Compound **5** (102 mg, 0.079 mmol) was dissolved in 3% KOH/MeOH (7.0 mL) and heated at 65 °C. After stirring for 20 h, the mixture was purified by column chromatography (MCI gel, $CHP_{20}P$; water, 250.0 mL; MeOH, 150.0 mL), and the organic solvent was concentrated under diminished pressure to yield **26** (51 mg, 94%) as a colorless oil. $[\alpha]_D$ −45.1 (*c* 0.05, MeOH); HRESIMS: calcd for $C_{18}H_{32}NaO_{14}$ 495.1690, found *m*/*z* 495.1485 $[M+Na]^+$; ^1H NMR (in C_5D_5N): δ 5.91 (1H, d, *J* 3.7 Hz, Glc H-1), 5.83 (1H, s, Rha H-1) 5.76 (1H, s, Rha′ H-1), 1.73 (3H, d, *J* 6.1 Hz, Rha H-6), 1.59 (3H, d, *J* 6.1 Hz, Rha′ H-6); ^{13}C NMR (in C_5D_5N): δ 96.5, 79.6, 75.5, 79.5, 78.9, 61.5 (Glc C-1–6), 103.1, 72.3, 72.8, 73.3, 70.4, 18.5 (Rha [1→2] C-1–6), 103.0, 72.4, 72.9, 74.0, 70.4, 18.6 (Rha [1→4] C-1–6).

3.12. Cytotoxicity bioassays[3a]

Growth inhibition experiments were carried out in quadruplicate in 96-well microplates, and the number of viable cells at the end of incubation period was determined using an MTT assay.[22] Each sample was dissolved in Me_2SO (2.0 mg/mL) and diluted with an experimental growth medium such that the final Me_2SO concentration was less than 0.5%. PC-12 and HCT116 cells were seeded in a 96-well microplate at a concentration of 1000 cells/well. They were continuously cultured without or with five concentrations (5000, 1000, 200, 40, and 8 ng/mL) of test compounds for 72 h starting from the next day. After adding MTT (20 μL, 5 mg/mL in phosphate-buffered saline; Sigma), the medium was removed, and the blue dye that formed was dissolved in 150 μL of Me_2SO. The absorbance was measured, and the *T*/*C* (%) score was calculated by the formulae described below. A graph of the concentration of samples against *T*/*C* (%) was plotted. The concentration value that corresponded with 50% *T*/*C* was designated as the GI_{50} value

$$T/C\ (\%) = (T-S)/(C-S)\times 100$$

where *T* is the OD_{550} value of the cell with the samples after a 3-d incubation, *C* the OD_{550} value of the cell without the samples, and *S* the OD_{550} value of the cell before the samples were added.

The GI_{50} value was defined as the concentration of sample necessary to inhibit the growth to 50% of the control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.06.008.

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