An efficient synthesis of julibrosides related to neosaponin

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A practical method is developed for the synthesis of neosaponin cholest-5(6)-en-3β-yl β-D-glucopyranosyl-(1⟶3)-[α-L-arabinofuranosyl-(1⟶4)]-α-L-rhamnopyranosyl-(1⟶2)-β-D-glucopyranoside. This julibroside mimic shows cytotoxic activity towards P388 (mouse leukaemia) with GI_{50} = 22.5 μg mL^{-1}.

Introduction

Albizia julibrissin is a plant widely distributed in China. The stem bark of the plant has been used as a sedative and anti-inflammatory drug to treat lung swellings and pains, skin ulcers, and wounds.[1] Julibrosides, isolated triterpenoid glycosides from *Albizia julibrissin*, show cytotoxicity against KB cells.[2] When their ester-linked tetrasaccharide was removed, the cytotoxicity dramatically decreased.[3] This finding prompted us to prepare neosaponins carrying the original tetrasaccharide component and to examine their biological activities.

Although the tetrasaccharide has been prepared by lithium iodide-promoted ester cleavage of julibrosides, its final glycosylation with an aglycone is still troublesome.[2b,4] Many reports revealed that coupling of the aglycone with a glycosyl donor without the participation of a neighboring group produced a mixture of α and β anomers, which were difficult to separate.[4,5] Our method is to connect the *endo*-glucose unit to the aglycone moiety at the very beginning in order to get exclusive β-linkage, then manipulate the glucose on C-2 and extend the sugar chain subsequently.[6]

Results and discussion

In our previous synthesis of 1⟶3-linked rhamnopyranoside,[7a] we showed that chemoselective deacetylation in the presence of the benzoyl group could be best controlled under acidic conditions.[8] Thus, orthoester **1b**[9] was treated with 95% aq. acetic acid,[10] followed by Schmidt activation[11] on the anomeric center using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), to give 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **2** (87% yield for two steps) (Scheme 1). Coupling of **2** with cholesterol **3** in anhydrous CH_2Cl_2 in the presence of TMSOTf (0.1 equiv.) furnished exclusively β-D-glucopyranoside **4** in 91% yield. The doublet at δ 4.79 with coupling constant of 7.9 Hz clearly showed the β linkage between the glucopyranosyl moiety and the cholesterol residue. It is noteworthy that attempts using the corresponding bromide as the donor generated orthoester as a significant by-product while the Helfrich reaction[12] of 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzoyl-β-D-glucopyranose with **3** gave only a 40% yield of desired compound **4**. Regioselective deacetylation of **4** with 3% acetyl chloride in MeOH–CH_2Cl_2 (1 : 1) co-solvent was smoothly carried out and 2-OH acceptor **5** was provided in 89% yield as a white solid.

Initial attempts at the synthesis of trisaccharide donor **18** followed our previous strategy of sugar–sugar orthoester formation–rearrangement. However, TMSOTf-catalyzed rearrangement of **6** was surprisingly unpractical for gram-scale preparation of **8b** since an unidentified by-product was obtained in significant quantities. Thus, the 4-OH of the rhamnopyranosyl residue in the disaccharide orthoester **6**[7a] was protected with the chloroacetyl group using chloroacetic anhydride in pyridine to give **7**, treatment of which with TMSOTf gave **8a** in 77% yield. Unfortunately, cleavage of the chloroacetyl group of **8a** was quite difficult and a low yield of **8b** was given although several reaction conditions were investigated, including the use of thiourea–$NaHCO_3$–EtOH, thiourea–lutidine–EtOH, thiourea–Et_3N–1,4-dioxane, and ethylenediamine–Pyr in this reaction.[13]

Alternatively, the α-L-arabinofuranosyl trichloroacetimidate **9**[7b] and α-L-rhamnopyranoside acceptor **10**[14] were coupled first under standard glycosylation conditions (0.05 equiv. of TMSOTf, CH_2Cl_2, 0 °C, 1 hour) to give disaccharide **11** in 83% yield. Hydrolysis of **11** with 90% trifluoroacetic acid (TFA) gave diol **12** in 88% yield. At this stage, 2,3-selective glycosylation on disaccharide **12** gave mainly 1⟶2-linked product.[7a] To overcome this problem, regioselective benzoylation of **12** was tried with 1.05 equiv. of BzCl in pyridine in an ice–water-bath, and 2-*O*-benzoylated product **13** was obtained in 79% yield as an amorphous solid. The chemical shifts of H-2 (δ 5.36, $J_{1,2}$ 1.4, $J_{2,3}$ 3.3 Hz) and H-3 (δ 4.39, $J_{2,3}$ 3.3, $J_{3,4}$ 9.3 Hz), based on 2D ^1H–^1H COSY analysis, assured the regioselectivity at C-2 of **12**. Coupling of fully acetylated glucopyranosyl trichloroacetimidate **14** with disaccharide acceptor **13** in anhydrous CH_2Cl_2 using a catalytic amount of TMSOTf (0.1 equiv.) at 0 °C for 2 hours gave trisaccharide **15** as the major product (63%), together with 20% of orthoester **16**. The orthoester formation was found to be dependent on the amount of promoter used in the reaction and could be well depressed by adding another portion of TMSOTf (0.03 equiv.). Thus, trisaccharide **15** was prepared in 78% yield without formation of detectable orthoester within 4 hours. Trisaccharide imidate **18** was prepared from **15** in two steps (deallylation to **17**: $PdCl_2$, NaOAc, HOAc; activation: Cl_3CCN, DBU, CH_2Cl_2) in a total yield of 55%. TMSOTf-catalyzed glycosylation of **18** and **5** in CH_2Cl_2 finished assembly of this neosaponin derivative **19** (86%) whose structure was determined with the help of ^1H NMR, ^{13}C NMR and ^1H–^1H, ^1H–^{13}C COSY spectra. Treatment of fully acyl-protected **19** with NaOMe in MeOH afforded the designed neosaponin **20** in 95% yield.

The cytotoxicity of the neosaponin **20** was preliminarily examined by incubation of the P388 cell line with **20** at 37 °C for 72 hours in a 5% CO_2 incubator.[15] The plate was read on a microplate reader at a wavelength of 540 nm. Neosaponin **20** showed cytotoxic activity on inhibition of P388 cell growth

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increase at GI_{50} 22.5 μg mL^{-1}. This result demonstrated Nohara's idea that the tetrasaccharide moiety of julibrosides plays an important role in the expression their cytotoxic activities.[2b] Synthesis of the tetrasaccharide-containing neosaponin analogues and the testing of their bioactivities are currently underway in our group.

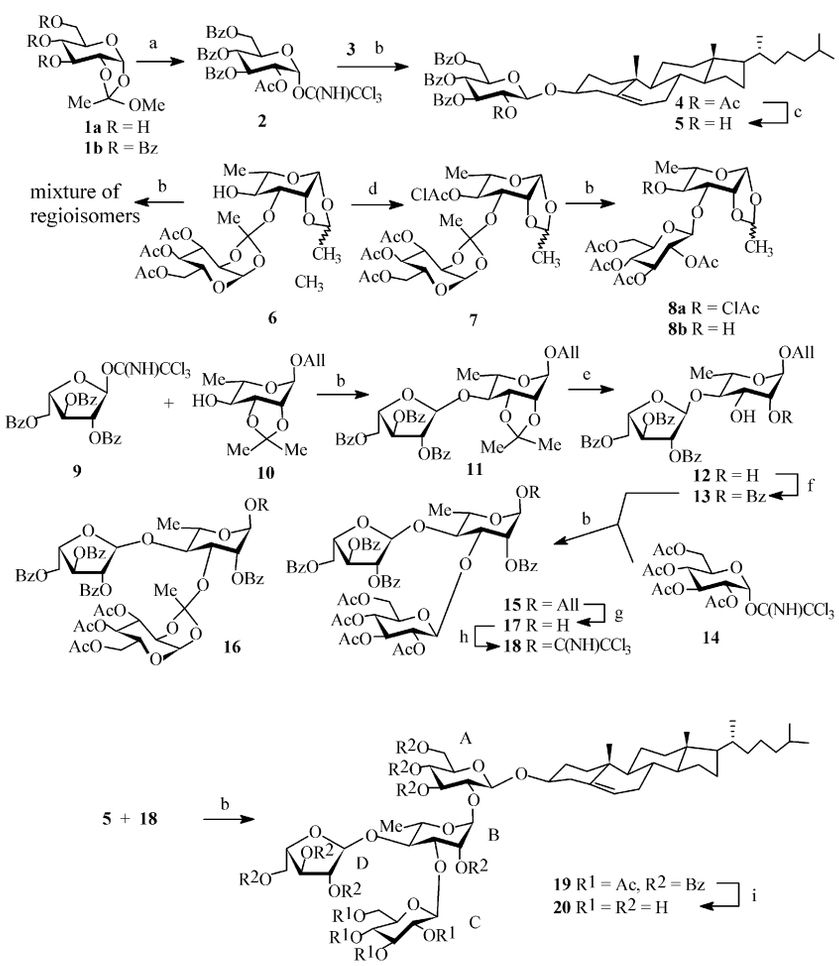
In summary, we present here a convergent synthesis of a julibroside-related neosaponin showing cytotoxic activity towards P388. This concise and practical synthetic strategy should provide the possibility for the synthesis of other analogues.

Experimental

Optical rotations were determined at 25 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter, and $[a]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. Mps were determined with a 'Mel-Temp' apparatus. ^1H NMR, ^{13}C NMR and ^1H–^1H, ^1H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or CD_3OD. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using a MALTI-TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix or recorded with a VG PLATFORM mass spectrometer using the ESI technique to introduce the sample. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detector. Column chromatography was conducted by elution of a column (10 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate 2

To a solution of compound **1a** (5.4 g, 22.9 mmol) in pyridine (20 mL) was added premixed BzCl–Pyr (3.2 mL of BzCl in 3 mL of pyridine) mixture under 0 °C. The reaction mixture was stirred at room temperature overnight, then poured into cold water and extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was combined, dried over anhydrous Na_2SO_4, and concentrated. The residue generated above was subjected to hydrolysis using 95% aq. acetic acid at room temperature for 15 min. The reaction mixture was poured into ice–water (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were successively washed with saturated aq. $NaHCO_3$ and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated and the residue was subsequently dissolved in anhydrous CH_2Cl_2 (15 mL). To the above solution were added trichloroacetonitrile (6 mL, 60 mmol) and DBU (0.6 mL) and the mixture was stirred at room temperature for 3 hours, then evaporated, and the residue was purified on a silica gel column (petroleum ether–EtOAc, 1.5 : 1) to give **2** (13.2 g, 85% from **1a**) as a white foam; $[a]_D^{25}$ +32.3 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.96 (s, 3 H, CH_3CO), 4.45 (dd, 1 H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 12.3 Hz, H^a-6), 4.55–4.63 (m, 2 H, H-5, H^b-6), 5.43 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.72 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 6.08 (t, 1 H, H-3), 6.69 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 7.26–8.02



Scheme 1 *Reagents and conditions (yields)*: a) 95% AcOH, Cl_3CCN, DBU, CH_2Cl_2 (87%); b) TMSOTS, CH_2Cl_2 (91% for **4**, 77% for **8a**, 83% for **11**, 78% for **15**, 86% for **19**); c) 3% acetyl chloride in MeOH–CH_2Cl_2 (1 : 1), (89%); d) $(ClCH_2CO)_2O$, Pyr (90%); e) 90% TFA (88%); f) BzCl, Pyr (79%); g) $PdCl_2$, NaOAc, HOAc; h) Cl_3CCN, DBU, CH_2Cl_2 (55% for two steps); i) NaOMe, MeOH (95%).

(m, 15 H, Ph), 8.72 (s, 1 H, C=NH) [MALDI-TOF-MS Calc. for $C_{31}H_{26}Cl_3NNaO_{10}$ (M + Na)$^+$: 700.05. Found: m/z, 700 (M + Na)$^+$].

Cholest-5-en-3β-yl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-β-D-glucopyranoside 4

Compound **2** (5.9 g, 8.7 mmol) and cholesterol **3** (3.32 g, 8.6 mmol) were dissolved in anhydrous dichloromethane (40 mL) at room temperature, and then TMSOTf (156 μL, 0.86 mmol) was added dropwise. The mixture was stirred under these conditions for 4 hours, at the end of which time TLC showed that the donor **2** was completely consumed. The reaction mixture was quenched with Et_3N (4 drops) and the mixture were evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1.5 : 1) to give **4** as a syrup (7.05 g, 91%); $[\alpha]_D^{25}$ −15 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.68–2.27 (m, CH_3CO and protons of cholesterol), 3.52 (m, 1 H, OCH), 4.06 (m, 1 H, H-5), 4.47 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 12.0 Hz, H^a-6), 4.56 (dd, 1 H, $J_{5,6b}$ 3.2 Hz, H^b-6), 4.79 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.23 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 5.35 (br d, 1 H, =CH), 5.55 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.71 (t, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 7.30–8.00 (m, 15 H, Ph) [ESIMS Calc. for $C_{56}H_{70}NaO_{10}$ (M + Na)$^+$: 925.5. Found: m/z, 925.6 (M + Na)$^+$].

Cholest-5-en-3β-yl 3,4,6-tri-*O*-benzoyl-β-D-glucopyranoside 5

To a solution of **4** (3.16 g, 3.5 mmol) in anhydrous CH_2Cl_2–CH_3OH (1 : 1, 50 mL) was added acetyl chloride (1.5 mL) at 0 °C. The mixture was sealed in a flask and stirred at room temperature. The reaction was detected by TLC until the starting material disappeared (*ca.* 24 hours), then the mixture was neutralized with pyridine, and concentrated to near dryness. The residue was passed through a short silica gel column to give **5** (2.68 g, 89%) as a white solid; $[\alpha]_D^{25}$ −6 (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.67–2.49 (m, protons of cholesterol), 3.57–3.63 (m, 1 H, OCH), 3.79 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.3 Hz, H-2), 4.03–4.08 (m, 1 H, H-5), 4.48 (dd, 1 H, $J_{5,6a}$ 6.2, $J_{6a,6b}$ 12.0 Hz, H-6), 4.55 (dd, 1 H, $J_{5,6b}$ 3.5 Hz, H-6), 4.66 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.35 (br d, 1 H, =CH), 5.52 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 5.71 (t, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 7.30–8.00 (m, 15 H, Ph) [ESIMS Calc. for $C_{54}H_{68}NaO_9$ (M + Na)$^+$]: 883.5. Found: m/z, 883 (M + Na)$^+$] (Calc. for $C_{54}H_{68}O_9$: C, 75.35; H, 7.91. Found: C, 75.68; H, 7.70%).

3′,4′,6′-Tri-*O*-acetyl-α-D-glucopyranose-1′,2′-diyl 4-*O*-chloroacetyl-1,2-*O*-((*R*,*S*)-ethylidene)-β-L-rhamnopyranos-3-yl orthoacetate 7

To a solution of **6** (790 mg, 1.5 mmol) in pyridine (3 mL) was added chloroacetic anhydride (342 mg, 2 mmol). The mixture was kept at room temperature for 3 hours, then poured into cold water (6 mL), and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phase was dried over anhydrous sodium sulfate. After filtration and concentration under reduced pressure, the obtained residue was purified by column chromatography (1 : 1.5 EtOAc–petroleum ether) to give **7** (815 mg, 90%). A small amount of pure *R*-isomer of **7** was obtained as crystals: mp 174–176 °C; $[\alpha]_D^{25}$ −65 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3 H, $J_{5,6}$ 6.3 Hz, H_3-6), 1.52 (d, 3 H, *J* 4.9 Hz, CH_3CH), 1.77 (s, 3 H, CH_3), 2.09, 2.10, 2.13 (3 s, 9 H, 3 × Ac), 3.52 (dq, 1 H, $J_{4,5}$ 9.4 Hz, H-5), 3.90 (m, 1 H, H-5′), 4.00 (dd, 1 H, $J_{2,3}$ 4.3, $J_{3,4}$ 9.4 Hz, H-3), 4.08, 4.14 (2 d, 2 H, *J* 14.7 Hz, $ClCH_2$), 4.21–4.26 (m, 2 H, H_2-6′), 4.24 (dd, 1 H, H-2), 4.44 (dd, 1 H, H-2′), 4.94 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.9 Hz, H-4′), 5.03 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 5.18 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.25 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1), 5.34 (q, 1 H, CH_3CH), 5.70 (d, 1 H, $J_{1',2'}$ 5.2 Hz, H-1′) (Calc. for $C_{24}H_{33}ClO_{15}$: C, 48.28; H, 5.53. Found: C, 48.46; H, 5.41%).

2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-4-*O*-chloroacetyl-1,2-*O*-((*R*,*S*)-ethylidene)-β-L-rhamnopyranose 8a

A suspension of **7** (620 mg, 1.04 mmol) containing 4 Å Molecular Sieves in anhydrous CH_2Cl_2 (20 mL) was cooled to −10 °C, then TMSOTf (10 μL, 0.05 mmol) was added under N_2 flow. The mixture was stirred at this temperature for about 1 hour, then neutralized with 2 drops of triethylamine. After filtration and concentration under reduced pressure, the obtained residue was purified by column chromatography (1 : 1.5 EtOAc–petroleum ether) to give **8a** as crystals (477 mg, 77%). Pure *R*-isomer gave the following physical data: mp 158–160 °C; $[\alpha]_D^{25}$ −35 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 3 H, $J_{5,6}$ 6.2 Hz, H_3-6), 1.53 (d, 3 H, *J* 4.9 Hz, CH_3CH), 2.00, 2.03, 2.05, 2.10 (4 s, 12 H, 4 × Ac), 3.49 (dq, 1 H, $J_{4,5}$ 9.5 Hz, H-5′), 3.70 (m, 1 H, H-5′), 4.00 (dd, 1 H, $J_{2,3}$ 4.0, $J_{3,4}$ 9.7 Hz, H-3), 4.10, 4.14 (2 d, 2 H, *J* 15 Hz, $ClCH_2$), 4.21–4.24 (m, 3 H, H-2, H_2-6′), 4.78 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 5.04 (t, 1 H, H-2′), 5.11 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 5.03 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 5.18 (t, 1 H, $J_{2',3'}$ 9.5 Hz, H-3′), 5.25 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.33 (q, 1 H, CH_3CH) (Calc. for $C_{24}H_{33}ClO_{15}$: C, 48.28; H, 5.53. Found: C, 48.37; H, 5.43%).

Allyl 2′,3′,5′-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside 11

To an ice–water-cooled solution of **9** (6.67 g, 11 mmol) and **10** (2.45 g, 10 mmol) in anhydrous CH_2Cl_2 (50 mL) was added TMSOTf (70 μL, 0.39 mmol). The mixture was stirred at this temperature for 2 hours, then quenched with Et_3N (4 drops). The mixture was evaporated *in vacuo* to give a residue, which was purified on a silica gel column chromatography (petroleum ether–EtOAc, 2 : 1) to give disaccharide **11** (5.73 g, 83%) as a syrup; $[\alpha]_D^{25}$ −11 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 3 H, CH_3), 1.31 (d, 3 H, H_3-6), 1.55 (s, 3 H, CH_3), 3.69 (dd, 1 H, *J* 7.2, 9.9 Hz, H-4), 3.76 (dq, 1 H, H-5), 4.00–4.21 (m, 3 H, H-2, CH_2=CHCH_2), 4.33 (dd, 1 H, *J* 7.0, 5.8 Hz, H-3), 4.59 (m, 1 H, H-4′), 4.7 (dd, 1 H, *J* 5.6, 11.8 Hz, H^a-5′), 4.8 (dd, 1 H, *J* 3.6 Hz, H^b-5′), 5.04 (s, 1 H, H-1), 5.20–5.35 (m, 2 H, CH_2=CHCH$_2$), 5.54 (d, 1 H, *J* 4.6 Hz, H-3′), 5.60 (s, 1 H, H-2′/H-1′), 5.81 (s, 1 H, H-1′/H-2′), 5.87–5.95 (m, 1 H, CH_2=CHCH_2), 7.28–8.09 (m, 15 H, Ph) (Calc. for $C_{38}H_{40}O_{12}$: C, 66.28; H, 5.81. Found: C, 66.04; H, 6.09%).

Allyl 2′,3′,5′-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-2-*O*-benzoyl-α-L-rhamnopyranoside 13

To a solution of compound **11** (4.79 g, 6.96 mmol) in CH_2Cl_2 (2 mL) was added 90% aq. trifluoacetic acid (10 mL). The mixture was stirred at room temperature for 20 min, then the mixture was evaporated *in vacuo* in the presence of toluene (2 × 15 mL) and the residue was purified on a short silica gel column (petroleum ether–EtOAc, 1 : 1) to give crude diol **12** as a syrup.

To an ice–water-cooled solution of **12** (3.1 g, 4.78 mmol) in pyridine (20 mL) was added benzoyl chloride (0.58 mL, 5.02 mmol). The mixture was stirred at this temperature for 6 hours, then poured into water and extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phase was dried over Na_2SO_4 and evaporated to give a syrup. Column chromatography gave pure **13** as an amorphous solid (3.7 g, 79%); $[\alpha]_D^{25}$ +13 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42 (d, 3 H, *J* 6.1 Hz, H_3-6), 3.79 (t, 1 H, *J* 9.3 Hz, H-4), 3.87 (dq, 1 H, H-5), 4.01–4.23 (m, 2 H, CH_2=CHCH_2), 4.39 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 4.65–4.71 (m, 2 H, H-4′ and H^a-5′), 4.80 (dd, 1 H, *J* 8.4 Hz, H^b-5′), 4.92 (d, 1 H, *J* 1.4 Hz, H-1), 5.21–5.36 (m, 2 H, CH_2=CHCH$_2$), 5.36 (dd, 1 H, H-2), 5.43 (d, 1 H, *J* 2.7 Hz, H-2′), 5.68–5.71 (m, 2 H, H-1′ and H-3′), 5.9–6.00 (m, 1 H, CH_2=CHCH$_2$), 7.25–8.09 (m, 20 H, Ph) [ESIMS calcd for $C_{42}H_{40}NaO_{13}$ (M + Na)$^+$: 775.2. Found: m/z, 775 (M + Na)$^+$]

(Calc. for $C_{42}H_{40}O_{13}$: C, 67.02; H, 5.32. Found: C, 66.90; H, 5.44%).

Allyl 2^{D},3^{D},5^{D}-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-[2^{C},3^{C},4^{C},6^{C}-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2^{B}-*O*-benzoyl-α-L-rhamnopyranoside 15

To a cooled solution (0 °C) of **13** (2.106 g, 2.8 mmol) and **14** (1.45 g, 2.94 mmol) in anhydrous CH_2Cl_2 (25 mL) was added TMSOTf (50 μL, 0.28 mmol). The mixture was stirred at this temperature for 40 min, then another portion of TMSOTf (15 μL, 0.08 mmol) was added. The mixture was stirred at 0 °C for 3 hours, then was quenched with Et_3N (4 drops). The mixture was evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1 : 1) to give trisaccharide **15** (2.36 g, 78%) as a syrup; $[\alpha]_D^{25}$ −1 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.37 (d, 3 H, *J* 6.4 Hz, H_3-6^{B}), 1.49, 1.82 (2 s, 2 × 3 H, 2 Ac), 2.04 (s, 6 H, 2 Ac), 3.84–3.90 (m, 2 H, H-5^{B}, H-5^{C}), 4.00 (t, 1 H, *J* 9.4 Hz, H-4^{B}), 4.05 (m, 1 H, one proton of CH_2=CHC*H*$_2$), 4.14–4.20 (m, 3 H, H_2-6^{C}, one proton of CH_2=CHC*H*$_2$), 4.42 (dd, 1 H, *J* 3.4, *J* 10.4 Hz, H-3^{B}), 4.65–4.70 (m, 2 H, H-4^{D}, H-1^{C}), 4.80–5.02 (m, 5 H, *J* 7.8, *J* 9.1, *J* 1.5 Hz, H_2-5^{D}, H-1^{B}, H-2^{C}, H-4^{C}), 5.21 (t, 1 H, *J* 9.3 Hz, H-3^{C}), 5.25 (m, 1 H, C*H*$_2$=CHCH$_2$), 5.31 (dd, 1 H, H-2^{B}), 5.34 (m, 1 H, C*H*$_2$=CHCH$_2$), 5.53 (d, 1 H, *J* 1.0 Hz, H-1^{D}), 5.65 (br d, 1 H, *J* 3.8 Hz, H-3^{D}), 5.77 (s, 1 H, H-2^{D}), 5.95 (m, 1 H, CH_2=CHCH$_2$), 7.26–8.11 (m, 20 H, Ph) (Calc. for $C_{56}H_{58}O_{22}$: C, 62.11; H, 5.36. Found: C, 62.38; H, 5.37%).

The by-product was elucidated to be the orthoester **16**; $[\alpha]_D^{25}$ −6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.41 (d, 3 H, *J* 5.5 Hz, H_3-6), 1.75 (s, 3 H, CH_3O), 1.94, 1.95, 2.07 (3 s, 3 × 3 H, 3 × Ac), 3.77–3.79 (m, 1 H), 3.87–3.90 (m, 2 H), 4.07 (dd, 1 H), 4.11 (m, 2 H), 4.15 (dd, 1 H), 4.18–4.23 (m, 1 H), 4.30 (dd, 1 H), 4.66–4.70 (m, 2 H), 4.74–4.78 (m, 2 H), 4.85 (d, 1 H), 4.99 (t, 1 H), 5.24–5.36 (m, 2 H), 5.48 (dd, 1 H), 5.55 (dd, 1H), 5.65 (d, 1 H), 5.71 (d, 1 H), 5.72 (s, 1 H), 5.90–5.99 (m, 1 H), 7.25–8.10 (m, 20 H, Ph).

2^{D},3^{D},5^{D}-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-[2^{C},3^{C},4^{C},6^{C}-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2^{B}-*O*-benzoyl-α-L-rhamnopyranosyl 2,2,2-trichloroacetimidate 18

To a solution of **15** (1.4 g, 1.29 mmol) in 90% AcOH (aq., 10 mL) were added NaOAc (497 mg, 5.2 mmol) and $PdCl_2$ (497 mg, 2.6 mmol). The mixture was stirred at room temperature for 16 hours, at the end of which time TLC (1 : 1 petroleum ether–EtOAc) showed the completion of the reaction. The mixture was diluted with CH_2Cl_2 (30 mL) and then filtered. The filtrate was neutralized with saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was combined and concentrated, then subjected to silica gel column chromatography (1 : 1 petroleum ether–EtOAc). The product generated above (**17**) was dissolved in CH_2Cl_2 (20 mL) and trichloroacetonitrile (1.0 mL, 10 mmol), and DBU (0.12 mL) was added. The mixture was stirred at room temperature for 3 hours. TLC (2 : 1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure and then purified on a silica gel column using 2 : 1 petroleum ether–EtOAc as eluent to give **18** as a syrup (842 mg, 55%); $[\alpha]_D^{25}$ −6 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.41 (d, 3 H, H_3-6^{B}), 1.44, 1.83, 2.02, 2.04 (4 s, 4 × 3 H, 4 × Ac), 3.84 (m, 1 H, H-5^{B}), 4.00–4.21 (m, 4 H, H-5^{C}, H-4^{B}, H_2-6^{C}), 4.49 (dd, 1 H, *J* 3.5, *J* 8.9 Hz, H-3^{B}), 4.70–4.75 (m, 2 H, H-1^{C}, H-4^{D}), 4.80, 4.90 (2 dd, 2 H, *J* 7.9, *J* 9.7, *J* 6.0 Hz, H_2-5^{D}), 5.01–5.09 (m, 2 H, *J* 8.0, *J* 9.3 Hz, H-2^{C}, H-4^{C}), 5.25 (t, 1 H, *J* 9.3 Hz, H-3^{C}), 5.47 (dd, 1 H, *J* 3.4, *J* 1.9 Hz, H-2^{B}), 5.58 (s, 1 H, H-1^{D}), 5.66 (d, 1 H, *J* 3.2 Hz, H-3^{D}), 5.73 (s, 1 H, H-2^{D}), 6.36 (d, 1 H, *J* 1.6 Hz, H-1^{B}), 7.25–8.08 (m, 20 H, Ph), 8.76 (s, 1 H, C=NH) (Calc. for $C_{55}H_{54}Cl_3NO_{22}$: C, 55.62; H, 4.55. Found: C, 55.41; H, 4.73%).

Cholest-5-en-3β-yl 2^{D},3^{D},5^{D}-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→4)-[2^{C},3^{C},4^{C},6^{C}-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2^{B}-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3^{A},4^{A},6^{A}-tri-*O*-benzoyl-β-D-glucopyranoside 19

Pre-dried compound **18** (1.15 g, 9.7 mmol) and glycosylated cholesterol derivative **5** (874 mg, 9.7 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL) in an ice–water-bath, and then TMSOTf (50 μL, 0.28 mmol) was added. The mixture was stirred at this temperature for 2 hours, at the end of which time TLC showed that the donor **18** was completely consumed. The reaction mixture was quenched with Et_3N (2 drops) and the mixture was evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1.5 : 1) to give **19** as a syrup (1.57 g, 86%); $[\alpha]_D^{25}$ +11 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.62, 1.81, 1.88, 1.98 (4 s, 12 H, 4 × Ac), 3.24 (dt, 1 H, *J* 2.5, 9.9 Hz, H-5^{C}), 3.59 (m, 1 H, H-3 of cholesterol), 3.90 (dd, 1 H, *J* 2.5, 12.4 Hz, H-6^{C}), 3.95–4.05 (m, 3 H, H-6^{C}, H-4^{B}, H-2^{A}), 4.09 (m, 1 H, H-5^{A}), 4.20–4.30 (m, 2 H, H-3^{B}, H-5^{B}), 4.50 (dd, 1 H, *J* 6.0, 11.2 Hz, H-6^{A}), 4.61 (dd, 1 H, *J* 7.9 Hz, H-1^{C}), 4.62–4.69 (m, 2 H, H-4^{D}, H-5^{D}), 4.74–4.77 (m, 2 H, *J* 7.8 Hz, H-1^{A}, H-5^{D}), 4.81 (dd, 1 H, *J* 7.9, 9.7 Hz, H-2^{C}), 4.90 (t, 1 H, H-4^{C}), 5.06 (t, 1 H, H-3^{C}), 5.08 (br s, 2 H, H-1^{B}, H-2^{B}), 5.32 (m, 1 H, =CH), 5.45 (br s, 1 H, H-1^{D}), 5.48 (t, 1 H, *J* 9.5 Hz, H-4^{A}), 5.56 (d, 1 H, *J* 3.9 Hz, H-3^{D}), 5.75 (s, 1 H, H-2^{D}), 5.80 (t, 1 H, H-3^{A}), 7.25–8.09 (m, 35 H, Ph); ^{13}C NMR ($CDCl_3$; 100 MHz) for sugar residues: δ_C 18.3 (C-6 of rhamnose), 60.9, 63.5, 63.9, 68.0, 70.1, 71.1, 71.2, 71.9, 72.8, 74.1, 74.9, 75.8, 77.2, 78.0, 78.5, 80.0, 81.9, 82.0 (C-2–C-5), 97.7 (C-1^{A}), 99.6 (C-1^{C}), 100.3 (C-1^{B}), 105.9 (C-1^{D}), 165.2, 165.4, 165.5, 165.6, 165.7, 166.0, 166.1 (7 × Ph*C*O), 168.5, 169.0, 169.9, 170.7 (4 × CH_3*C*O); ESIMS (positive) [calc. for $C_{107}H_{120}NaO_{30}$ (M + Na)$^+$: 1907.8. Found: *m*/*z*, 1908.2 (M + Na)$^+$] (Calc. for $C_{107}H_{120}O_{30}$: C, 68.15; H, 6.37. Found: C, 68.01; H, 6.44%).

Cholest-5-en-3β-yl α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside 20

Compound **19** (510 mg, 0.27 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and diluted with absolute methanol (45 mL). To the above mixture was added sodium methoxide (0.5 M in MeOH; 0.6 mL). The mixture was stirred at room temperature for 4 hours, then neutralized with Dowex 50 (H^+), filtered and the filtrate was concentrated under reduced pressure. The residue was purified by Sephadex LH-20 column chromatography (MeOH) to give **20** as a white solid (253 mg, 95%); $[\alpha]_D^{25}$ −65 (*c* 0.6, CH_3OH); ^{13}C NMR (CD_3OD; 100 MHz) δ_C 141.1 (C-5 of cholesterol), 120.1 (C-6 of cholesterol), 110.3, 105.7, 104.3, 100.8, (C-1 of sugar residues), 85.3, 84.4, 79.7, 79.2, 78.9, 78.4 (2 C), 78.1, 76.8, 75.4, 71.9, 71.2, 70.6, 69.4, 62.9, 62.5, 62.0; ESIMS (positive) [Calc. for $C_{50}H_{84}NaO_{19}$ (M + Na)$^+$: 1011.5. Found: *m*/*z*, 1011.6 (M + Na)$^+$].

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