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Note

Facile synthesis of cleistetroside-2, a partially acetylated oligorhamnoside from *Cleistopholis glauca* and *patens*

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Abstract—A tetrasaccharide, dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (cleistetroside-2), was synthesized via '2+2' convergent strategy. Sequential regioselective 3-*O*-glycosylation of isopropyl 1-thio-α-L-rhamnopyranoside (**4**) with 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl trichloroacetimidate (**8**), and isopropyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-1-thio-rhamnopyranoside (**10**) with dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**12**), greatly facilitate the target availability.

Keywords: Cleistetroside; Glycosylation; Rhamnoside; Antibacterial

Annonaceae is a family of large trees that grow in the rain forests of Africa. Extracts from the stem bark of this species are used to treat stomach pain, bronchial diseases and hepatitis. The root is believed to be a vermifuge, and the leaves are commonly employed for the treatment of fever.[1,2] From the stem bark of *Cleistopholis glauca* and the leaves of *Cleistopholis patens* (Annonaceae), a number of partially acetylated oligorhamnoside derivatives were isolated.[1–4] Among these compounds, cleistetroside-2 [dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside] was found to show significant in vitro antibacterial activity against Gram-positive bacteria.[4] For investigation of the oligosaccharide's structure–bioactivity relationships in respect to antibacterial activities,[5] we have prepared a series of L-rhamnose oligosaccharides.[6] Here we would like to report a facile synthesis of cleistetroside-2, a partially acetylated oligorhamnoside isolated from the stem bark of *Cleistopholis glauca* and the leaves of *Cleistopholis patens* (Annonaceae).

As outlined in Scheme 1, fully acetylated L-rhamopyranose **1** was converted into dodecanyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**2**) by a method similar to that of reported.[7] Similarly, compound **1** was transformed into isopropyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**3**) under modified Helferich reaction conditions.[8] Deacetylation of **3** with NaOMe in methanol gave the acceptor, isopropyl 1-thio-α-L-rhamnopyranoside (**4**). 2,3-*O*-Isopropylidenation of **4** with $Me_2C(OMe)_2$ in the presence of TsOH in acetone, and acetylation with Ac_2O in pyridine, afforded isopropyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (**5**) in 85.5% yield over three steps. Cleavage of the isopropylidene group from **5** using aq 80% AcOH at 55 °C gave isopropyl 4-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**6**), which was then silylated with TBSCl and imidazol in DMF at 50 °C to give the building block, isopropyl 4-*O*-acetyl-2,3-di-*O*-*tert*-butyldimethylsilyl-1-thio-α-L-rhamnopyranoside (**7**) in 89% yield. Glycosyl donor 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl trichloroacetimidate (**8**) was obtained from **5**

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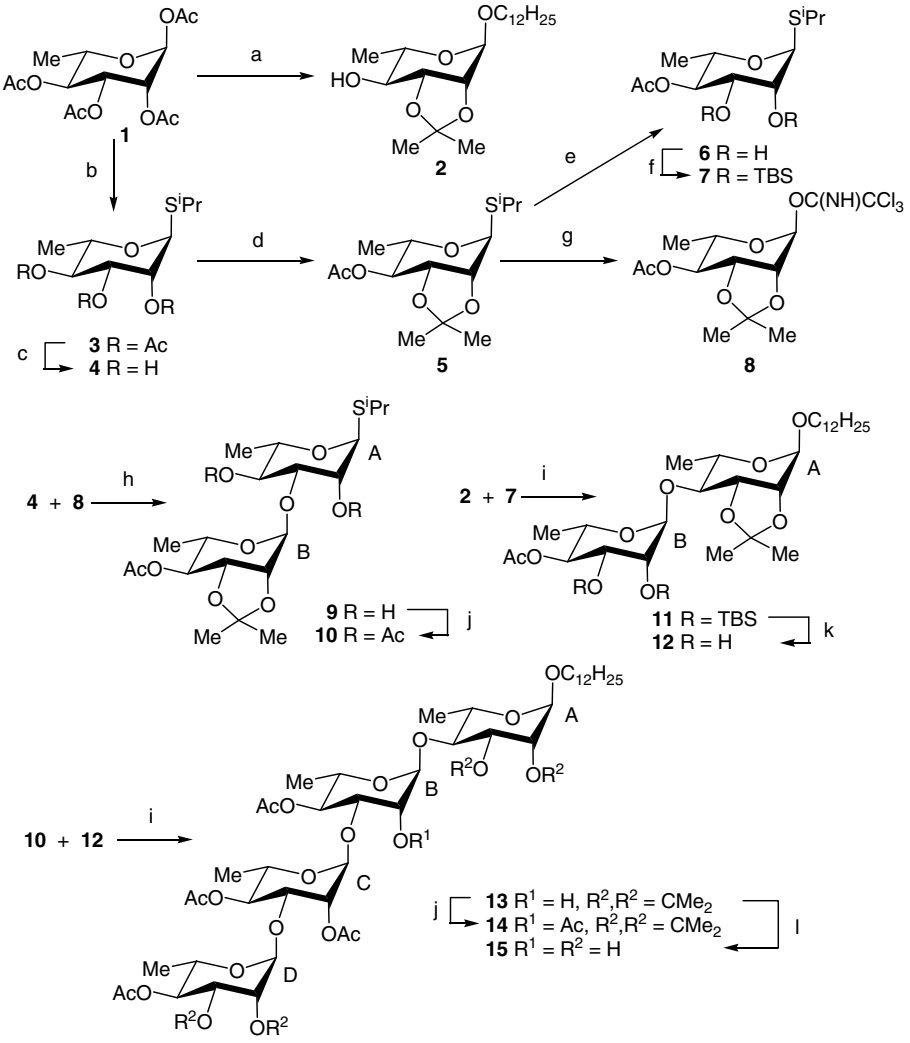

Scheme 1. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, $C_{12}H_{25}OH$, CH_2Cl_2, 83%; NaOMe, MeOH, 95%; TsOH, $Me_2C(OMe)_2$, 89%; (b) $BF_3{\cdot}Et_2O$, $HSCHMe_2$, CH_2Cl_2, 95%; (c) NaOMe, MeOH; (d) TsOH, $Me_2C(OMe)_2$; Ac_2O, Pyr, 90% for two steps; (e) 80% AcOH, 55 °C; (f) TBSCl, imidazole, DMF, 50 °C, 89% for two steps; (g) NBS, 9:1 acetone–H_2O; Cl_3CCN, DBU, CH_2Cl_2; (h) TMSOTf, CH_2Cl_2, 75%; (i) NIS, TMSOTf, CH_2Cl_2, 90% for **11**, 65% for **13**; (j) Ac_2O, Pyr, quantitative for **10**, 97% for **14**; (k) TBAF, THF, 70%; (l) 80% AcOH, reflux, 91%.

according to a published procedure.[7,9] The structure of **8** was assigned initially from its ^1H NMR spectrum and is further supported by its single-crystal X-ray structure (Fig. 1, Table 1).

Regioselective glycosylation of building blocks **4** and **8** in the presence of TMSOTf in anhyd CH_2Cl_2 afforded isopropyl 4′-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-α-L-1-thio-rhamnopyranoside (**9**) with complete α selectivity, in accordance with our previous report.[6] Side reactions, that is, thio-group transfer and sugar ring contraction, were not observed in this case.[10] The regioselectivity in the making of **9** was completely supported by its acetylated derivative, isopropyl 4′-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-1-thio-rhamnopyranoside (**10**). Peaks at δ 5.20 ppm (dd, 1H, J 1.4, 3.2 Hz, H-2^A) and 5.08 ppm (t, 1H, J 9.8 Hz, H-4^A) in the corresponding NMR spectra of **10** confirmed the (1→3)-linkage in its precursor **9**. Convergently, coupling of building blocks **2**[7] and **7** using NIS-TMSOTf as catalysts in dry CH_2Cl_2 gave (1→4)-linked disaccharide **11**, which was subjected to desilylation with TBAF[11] in THF, generating disaccharide acceptor, dodecanyl 4′-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**12**) in 63% yield for two steps. A regioselective glycosylation[6,12] of **10** and **12** was carried out smoothly using NIS and TMSOTf as catalysts in dry CH_2Cl_2 at −20 °C affording desired tetrasaccharide **13** in 65% isolated yield. The regioselectivity was postulated by NMR spectral analyses of acetylated **14**, which showed a downfield shift of a double-of-doublet at δ 5.22 ppm corresponding to H-2^C, which supports a newly formed (1→3)-linkage in **13**. Furthermore, H-1^C (δ 5.40 ppm, J <1.0 Hz), C-1^C (δ 99.4 ppm), and $^1J_{C,H}$ (167 Hz) of **13**

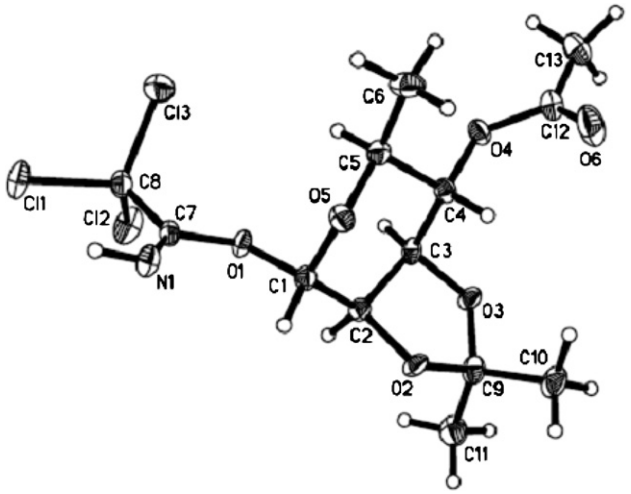

Figure 1. X-ray crystal structure of **8**. Thermal ellipsoids are shown on the 50% probability level.

Table 1. Selected bond lengths (Å) and bond angles (°) for glycosyl donor **8**

Bond lengths			
Cl(1)–C(8)	1.770(2)	Cl(2)–C(8)	1.772(2)
Cl(3)–C(8)	1.765(2)	O(1)–C(1)	1.450(2)
O(1)–C(7)	1.356(3)	O(2)–C(2)	1.432(3)
O(2)–C(9)	1.436(3)	O(3)–C(3)	1.437(3)
O(3)–C(9)	1.441(3)	O(4)–C(4)	1.447(3)
O(4)–C(12)	1.355(2)	O(5)–C(1)	1.391(2)
O(5)–C(5)	1.455(3)	O(6)–C(12)	1.199(3)
N(1)–C(7)	1.249(3)	C(1)–C(2)	1.521(3)
C(2)–C(3)	1.522(3)	C(3)–C(4)	1.526(3)
C(4)–C(5)	1.526(3)	C(5)–C(6)	1.504(4)
C(7)–C(8)	1.532(3)	C(9)–C(10)	1.511(3)
C(9)–C(11)	1.524(4)	C(12)–C(13)	1.495(4)
Bond angles			
C(1)–O(1)–C(7)	116.6(2)	C(2)–O(2)–C(9)	105.59(19)
C(3)–O(3)–C(9)	108.63(19)	C(4)–O(4)–C(12)	116.9(2)
C(1)–O(5)–C(5)	114.2(2)	O(1)–C(1)–O(5)	110.3(2)
O(1)–C(1)–C(2)	103.1(2)	O(5)–C(1)–C(2)	114.28(17)
O(2)–C(2)–C(1)	109.1(2)	O(2)–C(2)–C(3)	101.66(17)
C(1)–C(2)–C(3)	115.9(2)	O(3)–C(3)–C(2)	102.0(2)
O(3)–C(3)–C(4)	111.29(17)	C(2)–C(3)–C(4)	111.2(2)
O(4)–C(4)–C(3)	106.8(2)	O(4)–C(4)–C(5)	109.8(2)
C(3)–C(4)–C(5)	110.64(18)	O(5)–C(5)–C(4)	107.2(2)
O(5)–C(5)–C(6)	106.0(2)	C(4)–C(5)–C(6)	114.21(19)
O(1)–C(7)–N(1)	124.1(2)	O(1)–C(7)–C(8)	107.4(2)
N(1)–C(7)–C(8)	128.5(2)	Cl(1)–C(8)–Cl(2)	108.08(12)
Cl(1)–C(8)–Cl(3)	108.86(15)	Cl(1)–C(8)–C(7)	110.36(19)
Cl(2)–C(8)–Cl(3)	110.80(15)	Cl(2)–C(8)–C(7)	110.67(19)
Cl(3)–C(8)–C(7)	108.06(15)	O(2)–C(9)–O(3)	105.8(2)
O(2)–C(9)–C(10)	108.4(2)	O(2)–C(9)–C(11)	110.74(19)
O(3)–C(9)–C(10)	110.51(19)	O(3)–C(9)–C(11)	108.1(2)
C(10)–C(9)–C(11)	113.1(2)	O(4)–C(12)–O(6)	123.1(2)
O(4)–C(12)–C(13)	111.2(2)	O(6)–C(12)–C(13)	125.7(2)

confirmed its α configuration. Refluxing of **13** in aq 80% AcOH furnished cleistetroside-2 (**15**) in 91% yield.

The synthetic cleistetroside-2 (**15**) was bioassayed for its in vitro antibacterial activity against both Gram-negative and Gram-positive bacteria. A standard testing method was used following the instruction from the lit.[4,13] The preliminary results were summarized in Table 2.

Table 2. Antibacterial activity of the synthetic cleistetroside-2 (**15**)

Organism	MIC (μg/mL)
Escherichia coli ATCC 25922	>32
Escherichia coli clinical strains 25922	>32
Klebsislla pneumoniae ATCC 46117	16
Klebsislla pneumoniae clinical strains 46117	16
Pseudomonas aeruginosa ATCC 10211	32
Pseudomonas aeruginosa clinical strains 10211	32
Staphylococcus ATCC 25923	8
Staphylococcus clinical isolates 25923	8

In conclusion, a facile synthesis of natural cleistetroside-2 was achieved via '2+2' strategy taking advantage of the double-use regioselective glycosylation at C-3 of rhamnopyranose residues. This result would provide a practical approach to other partially acetylated oligorhamonoside analogues.[1–4]

1. Experimental

1.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR, ^{13}C NMR and COSY, and HMBC spectra were recorded with Bruker ARX 400 spectrometers for solutions in $CDCl_3$. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured with Q-TOF 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 a UV detector. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

1.2. Isopropyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (3)

To a solution of compound **1** (40 g, 120.4 mmol) and 2-propanethiol (14.7 mL, 156.5 mmol) in dry CH_2Cl_2 (160 mL) was added $BF_3{\cdot}Et_3O$ (45.5 mL, 361 mmol). The mixture was stirred at 0 °C for 30 min, and the temperature was then gradually raised to ambient temperature. The mixture was stirred at these conditions for 2 h, then diluted with CH_2Cl_2 (100 mL), washed with water (3×100 mL), neutralized by satd aq $NaHCO_3$, and washed with brine. The organic layers were combined, dried, and concentrated to give a residue. Purification

of the residue by column chromatography on silica gel (3:1 petroleum ether–EtOAc) gave compound **3** (39.8 g, 95%) as an amorphous solid: $[\alpha]_D^{25}$ −116 (*c* 3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.32 (dd, 1H, *J* 1.6, 3.3 Hz, H-2), 5.25 (d, 1H, *J* 1.6 Hz, H-1), 5.20 (dd, 1H, *J* 3.3, 9.8 Hz, H-3), 5.01 (t, 1H, *J* 9.8 Hz, H-4), 4.19–4.21 (m, 1H, H-5), 3.03–3.09 (m, 1H, $(CH_3)_2CH$), 2.18, 2.06, 1.98 (3s, 9H, 3 CH_3CO), 1.32 (d, 6H, *J*6.7 Hz, $(CH_3)_2CHS$), 1.22 (d, 3H, *J* 6.2 Hz, H-6). Anal. Calcd for $C_{15}H_{24}O_7S$: C, 51.71; H, 6.94. Found: C, 51.39; H, 6.88.

1.3. Isopropyl 4-*O*-acetyl-2,3-di-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (5)

A solution of **3** (11.45 g, 32.9 mmol) in MeOH (120 mL) was treated with NaOMe (5.0 mL, 1 M in MeOH) at rt for 3 h. The mixture was neutralized with IR-120 (H^+) resin, then filtered, and the filtrate was evaporated to give syrupy **4**. To this amount of **4** in acetone (60 mL) was added 2,2-dimethoxypropane (6.1 mL, 49.3 mmol) and *p*-toluenesulfonic acid monohydrate (300 mg) at 0 °C. The mixture was stirred at rt for 1 h, then neutralized with Et_3N (15 mL) and concentrated. The residue was treated with Ac_2O (20 mL) in pyridine (40 mL) at rt for 10 h, then co-evaporated with toluene under reduced pressure to dryness. Purification of the residue by column chromatography on silica gel (6:1 petroleum ether–EtOAc) gave compound **5** (9.0 g, 90%) as a syrup: $[\alpha]_D^{25}$ −143 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.59 (s, 1H, H-1), 4.91 (dd, 1H, *J* 7.8, 10.0 Hz, H-4), 4.19 (d, 1H, *J* 5.4 Hz, H-2), 4.09–4.18 (m, 2H, H-3, H-5), 3.02–3.08 (m, 1H, $(CH_3)_2CH$), 2.11 (s, 3H, CH_3CO), 1.57, 1.34 (2 s, 6H, $(CH_3)_2C$), 1.35, 1.30 (2 d, 6H, *J* 6.7 Hz, $(CH_3)_2CHS$), 1.16 (d, 3H, *J* 6.3 Hz, H-6). Anal. Calcd for $C_{14}H_{24}O_5S$: C, 55.24; H, 7.95. Found: C, 55.57; H, 7.87.

1.4. Isopropyl 4-*O*-acetyl-2,3-di-*O*-*tert*-butydimethylsilyl-1-thio-α-L-rhamnopyranoside (7)

A solution of compound **5** (5.00 g, 16.4 mmol) in AcOH (80 mL) and H_2O (20 mL) was stirred at 55 °C for 3 h, then evaporated with toluene to dryness under reduced pressure. The residue was dissolved in *N*,*N*′-dimethylformamide (DMF, 15 mL), imidazole (1.68 g, 24.7 mmol) and TBSCl (5.5 g, 36.2 mmol) were added at 0 °C. The mixture was then stirred at 50 °C for 12 h, diluted with water (200 mL) and extracted with EtOAc (3 × 200 mL). The organic phase was dried over anhyd Na_2SO_4 and concentrated. Purification of the residue on a silica gel column (20:1 petroleum ether–EtOAc) gave **7** (7.2 g, 89%) as a foam: $[\alpha]_D^{25}$ −61 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.11–5.06 (m, 2H, H-1 and H-4), 4.04–3.96 (m, 1H, H-5), 3.83 (br s, 1H, H-2), 3.83 (dd, 1H, *J* 2.6, 9.4 Hz, H-3), 3.06–3.00 (m, 1H, $(CH_3)_2CHS$), 2.03 (s, 3H, CH_3CO), 1.30 (d, 6H, *J* 4.7 Hz, $(CH_3)_3C$), 1.13 (d, 3H, *J* 6.5 Hz, H-6), 0.90, 0.85 (2s, 18H, ^{t}Bu), 0.08, 0.07, 0.06, 0.04 (4s, 12H, CH_3Si). Anal. Calcd for $C_{23}H_{48}O_5SSi_2$: C, 56.05; H, 9.82. Found: C, 56.59; H, 9.75.

1.5. Isopropyl 4-*O*-acetyl-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (10)

To a solution of **8** (4.91 g, 12.6 mmol) and **4** (2.53 g, 11.4 mmol) in dry CH_2Cl_2 (50 mL) was added TMSOTf (109 μL, 0.6 mmol) at 0 °C under an N_2 atmosphere. The mixture was stirred under these conditions for 40 min, and then quenched with Et_3N. The solvents were evaporated in vacuo to give a residue that was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give disaccharide **9**. Product **9** in pyridine (15 mL) and Ac_2O (3 mL) was stirred at rt for 2 h, then co-evaporated with toluene to dryness to give **10** (4.56 g, 75% for two steps) as an amorphous solid: $[\alpha]_D^{25}$ −45 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.22 (d, 1H, *J* 1.4 Hz, H-1^A), 5.20 (dd, 1H, *J* 1.4, 3.2 Hz, H-2^A), 5.08 (t, 1H, *J* 9.8 Hz, H-4^A), 5.07 (br s, 1H, H-1^B), 4.79 (dd, 1H, *J* 8.0, 10.2 Hz, H-4^B), 4.13–4.15 (m, 1H, H-5^A), 4.06–4.08 (m, 2H, H-3^A, H-3^B), 4.00 (br d, 1H, *J* 5.4 Hz, H-2^B), 3.65–3.69 (m, 1H, H-5^B), 3.03–3.06 (m, 1H, $(CH_3)_2CH$), 2.16, 2.09, 2.03 (3s, 9H, CH_3CO), 1.55, 1.31 (2s, 6H, $(CH_3)_2C$), 1.30 (d, 6H, $(CH_3)_2CHS$), 1.11, 1.22 (2d, 6H, *J* 6.3 Hz, H-6^A and H-6^B); ^{13}C NMR (100 MHz): δ 16.6, 17.2, 20.8, 20.9, 21.0, 23.4, 23.6, 26.2, 27.5, 30.8, 36.3, 64.7, 67.1, 73.2, 73.8, 74.0, 74.5, 75.4, 75.9, 81.5, 163.6, 170.0, 170.1, 170.3. Anal. Calcd for $C_{24}H_{38}O_{11}S$: C, 53.92; H, 7.16. Found: C, 54.30; H, 7.28.

1.6. Dodecanyl 4-*O*-acetyl-2,3-di-*O*-*tert*-butyldimethylsilyl-α-L-rhamno pyranosyl-(1→4)-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (11)

To a solution of **2** (3.22 g, 8.62 mmol) and **7** (4.77 g, 9.68 mmol) in anhyd CH_2Cl_2 (60 mL) was added NIS (2.33 g, 11.6 mmol) and TMSOTf (155 μL, 0.86 mmol) at 0 °C under an N_2 atmosphere. The reaction mixture was stirred under these conditions for 40 min, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (20:1 petroleum ether–EtOAc) to give **11** (6.12 g, 90%) as a syrup: $[\alpha]_D^{25}$ −50 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.12 (d, 1H, *J* 1.7 Hz, H-1^B), 5.08 (t, 1H, *J* 9.6 Hz, H-4^B), 4.94 (s, 1H, H-1^A), 4.12–4.07 (m, 2H, H-2^A, H-3^A), 3.88–3.83 (m, 2H, H-2^B, H-3^B), 3.71–3.62 (m, 3H, H-5^B, H-4^A, OCH_aH_b), 3.45–3.4 (m, 2H, H-5^A, OCH_aH_b), 2.04 (s, 3H, CH_3CO), 1.60–1.57 (m, 2H, OCH_2CH_2), 1.50, 1.31 (2s, 6H, $(CH_3)_2C$), 1.30–1.26 (m, 21H, CH_2 and H-6^A), 1.12 (d, 3H, *J* 6.4 Hz, H-

6^B), 0.91–0.85 (m, 21H, CH_3), 0.09, 0.08, 0.07, 0.06 (4s, 12H, CH_3Si). Anal. Calcd for $C_{41}H_{80}O_{10}Si_2$: C, 62.39; H, 10.22. Found: C, 62.01; H, 10.31.

1.7. Dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (12)

To a solution of **11** (6.53 g, 8.28 mmol) in THF (80 mL) was added TBAF (1.0 M THF solution, 25 mL, 25 mmol) at 0 °C. The mixture was stirred at these conditions for 30 min, then quenched with aq NH_4Cl. The water layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhyd Na_2SO_4, and concentrated in vacuo. The reaction mixture was purified by silica-gel column chromatography (1:1 petroleum ether–EtOAc) to give **12** (3.25 g, 70%) as an amorphous solid: $[\alpha]_D^{25}$ −72 (*c* 1.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 5.40 (d, 1H, *J* 1.3 Hz, H-1^B), 4.95 (s, 1H, H-1^A), 4.84 (t, 1H, *J* 9.7 Hz, H-4^B), 4.17 (dd, 1H, *J* 5.6, 7.2 Hz, H-4^A), 4.08 (d, 1H, *J* 5.6 Hz, H-3^A), 3.96 (s, 1H, H-2^A), 3.82–3.78 (m, 2H, H-5^B, H-2^B), 3.69–3.64 (m, 2H, H-5^A, OCH_aH_b), 3.50 (dd, 1H, *J* 7.3, 9.7 Hz, H-3^B), 3.42–3.40 (m, 1H, OCH_aH_b), 3.06 (d, *J* 7.3 Hz, O*H*), 2.99 (br s, 1H, O*H*), 2.13 (s, 3H, CH_3CO), 1.59–1.56 (m, 2H, OCH_2CH_2), 1.54, 1.33 (2s, 6H, $(CH_3)_2C$), 1.30–1.26 (m, 21H), 1.19 (d, 1H, *J* 6.3 Hz, H-6^B), 0.87 (t, 3H, *J* 7.0 Hz, CH_3). ^{13}C NMR (100 MHz): δ 14.0, 17.3, 17.9, 21.0, 22.6, 26.1, 26.3, 27.8, 29.2, 29.3, 29.5, 29.55, 29.57, 31.8, 63.8, 66.3, 67.6, 69.9, 71.1, 74.9, 76.2, 77.4, 78.4, 96.7, 98.1, 109.3, 171.6. Anal. Calcd for $C_{29}H_{52}O_{10}$: C, 62.12; H, 9.35. Found: C, 61.94; H, 9.41.

1.8. Dodecanyl 4-*O*-acetyl-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (13)

To a mixture of **10** (2.10 g, 3.93 mmol) and **12** (1.625 g, 2.9 mmol) in anhyd CH_2Cl_2 (40 mL) were added NIS (1.0 g, 4.35 mmol) and TMSOTf (80 μL, 0.44 mmol) at −20 °C under an N_2 atmosphere. The reaction mixture was stirred under these conditions for 30 min, then quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give **13** (1.92 g, 65%) as a white foam: $[\alpha]_D^{25}$ −55 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.39 (d, 1H, *J* 1.3 Hz, H-1^C), 5.10 (s, 1H, H-1^D), 5.07–5.05 (m, 2H, H-4^B, H-4^C), 4.99 (dd, 1H, *J* 1.7, 3.2 Hz, H-2^C), 4.95 (s, 1H, H-1^A), 4.88 (d, 1H, *J* 1.3 Hz, H-1^B), 4.80 (dd, 1H, *J* 8.1, 9.9 Hz, H-4^D), 4.16–4.06 (m, 4H, H-2^A, H-3^A, H-4^A, H-2^B), 4.02–3.94 (m, 3H, H-2^D, H-5^D, H-3^C), 3.87 (dd, 1H, *J* 3.1, 9.8 Hz, H-3^B), 3.82–3.78 (m, 1H, H-5^B), 3.70–3.60 (m, 3H, H-5^A, H-5^C, OCH_aCH_b), 3.50 (dd, 1H, *J* 7.3, 9.9 Hz, H-3^D), 3.43–3.39 (m, 1H, OCH_aH_b), 2.17, 2.11, 2.10, 2.09 (4s, 4 × 3H, 4 CH_3CO), 1.59–1.56 (m, 2H, OCH_2CH_2), 1.53 (s, 6H, 2 CH_3), 1.37–1.26 (m, 27H), 1.20, 1.18, 1.13 (3 d, 3 × 3H, *J* 6.4 Hz, 3 H-6), 0.87 (t, 3H, *J* 7.0 Hz, CH_3). ^{13}C NMR (100 MHz): δ 14.0, 16.4, 17.3, 17.4, 17.9, 20.6, 20.7, 20.9, 21.0, 22.6, 26.1, 26.2, 27.5, 27.8, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 63.6, 64.7, 66.7, 67.2, 67.7, 70.9, 71.7, 71.9, 72.5, 74.0, 74.2, 75.4, 75.9, 76.2, 77.8, 78.3, 78.4, 96.7, 97.9, 98.9, 99.4, 109.4, 109.6, 169.9, 169.98, 170.0, 170.3. HRESIMS: calcd for $C_{50}H_{82}O_{21}$ 1018.5349; found, 1041.5274 $[M+Na]^+$.

1.9. Dodecanyl 4-*O*-acetyl-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-*O*-isopropylidene-α-L-rhamnopyranoside (14)

To a solution of compound **13** (100 mg, 0.1 mmol) in pyridine (3 mL) was added DMAP (40 mg) and Ac_2O (2 mL). The mixture was stirred at 40 °C for 5 h, then evaporated with toluene to dryness under reduced pressure. Purification of the residue by column chromatography on silica gel (3:1 petroleum ether–EtOAc) gave **14** (101 mg, 97%) as a white foam: $[\alpha]_D^{25}$ −60 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.24 (d, 1H, *J* 1.6 Hz, H-1^C), 5.22 (dd, 1H, *J* 1.6, 3.1 Hz, H-2^C), 5.07 (t, 1H, *J* 6.4 Hz, H-4^C), 5.05 (s, 1H, H-1^D), 5.02 (t, 1H, *J* 10.0 Hz, H-4^B), 4.92 (s, 1H, H-1^A), 4.90 (dd, 1H, *J* 1.7, 3.2 Hz, H-2^B), 4.86 (d, 1H, *J* 1.7 Hz, H-1^B), 4.78 (dd, 1H, *J* 8.1, 10.0 Hz, H-4^D), 4.15 (dd, 1H, *J* 5.7, 7.1 Hz, H-4^A), 4.07 (d, 1H, *J* 5.5 Hz, H-2^A), 4.05 (dd, 1H, *J* 5.5, 7.1 Hz, H-3^A), 4.00–3.96 (m, 3H, H-2^D, H-3^B, H-3^C), 3.89–3.86 (m, 1H, H-5^B), 3.79–3.75 (m, 1H, H-5^C), 3.67–3.59 (m, 3H, H-5^A, H-5^D, OCH_aH_b), 3.45 (dd, 1H, *J* 7.3, 9.9 Hz, H-3^D), 3.42–3.39 (m, 1H, OCH_aH_b), 2.16, 2.12, 2.09, 2.08, 2.07 (5s, 5 × 3H, 5 CH_3CO), 1.57–1.55 (m, 2H, OCH_2CH_2), 1.51, 1.50, 1.30, 1.29 (4s, 4 × 3H, 2$(CH_3)_2C$), 1.26 (d, *J* 6.4 Hz, H-6^A), 1.24 (s, 18H, 9CH_2), 1.17, 1.16, 1.09 (3 d, 3 × 3H, *J* 6.4 Hz, H-6^B, H-6^C, H-6^D), 0.85 (t, 3H, *J* 7.0 Hz, CH_3). ESIMS: calcd for $C_{52}H_{84}O_{22}$ 1060.5; found, 1083.3 $[M+Na]^+$.

1.10. Dodecanyl 4-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (15)

To a solution of compound **13** (500 mg, 0.49 mmol) in AcOH (8 mL) and H_2O (2 mL) was stirred under refluxing overnight, then evaporated with toluene to dryness under reduced pressure. The residue was purified by silica gel column chromatography (5:1 EtOAc–MeOH) to give **15** (422 mg, 91%) as a gummy solid: $[\alpha]_D^{25}$ −64 (*c* 1, MeOH), [lit. $[\alpha]_D^{20}$ −59.5 (*c* 0.4, MeOH),[4] $[\alpha]_D^{20}$ −63.9 (*c* 0.4, MeOH)[7]]; ^1H NMR (400 MHz, $CDCl_3$): δ 5.35 (s,

1H, H-1^{B}), 5.12 (br s, 1H, H-2^{C}), 5.10 (t, 1H, *J* 9.7 Hz, H-4^{B}), 5.07 (t, 1H, *J* 9.7 Hz, H-4^{C}), 4.98 (br s, 1H, H-1^{C}), 4.88 (s, 1H, H-1^{D}), 4.78 (t, 1H, *J* 9.7 Hz, H-4^{D}), 4.72 (s, 1H, H-1^{A}), 4.28 (dd, 1H, *J* 2.8, 9.7 Hz, H-3^{C}), 4.11 (br s, 1H, H-2^{B}), 4.04–4.00 (m, 1H, H-5^{C}), 3.95–3.83 (m, 5H, H-2^{A}, H-3^{A}, H-2^{D}, H-3^{B}, H-5^{B}), 3.73 (dd, 1H, *J* 2.7, 9.7 Hz, H-3^{D}), 3.67–3.60 (m, 3H, H-5^{A}, H-5^{D}, OCH_aH$_b$), 3.53 (t, 1H, *J* 8.9 Hz, H-4^{A}), 3.38–3.35 (m, 1H, OCH$_a$$H_b$), 2.16, 2.15, 2.08, 2.05 (4s, 4 × 3H, 4 CH_3CO), 1.57–1.54 (m, 2H, OCH$_2$CH_2), 1.28 (d, 3H, *J* 6.5 Hz, H-6^{A}), 1.26 (s, 18H, 9CH$_2$), 1.22 (d, 3H, *J* 6.4 Hz, H-6^{C}), 1.18 (d, 3H, *J* 6.4 Hz, H-6^{B}), 1.10 (d, 3H, *J* 6.2 Hz, H-6^{D}), 0.88 (t, 3H, *J* 7.0 Hz, CH_3). ^{13}C NMR (100 MHz): δ 14.0, 17.0, 17.3, 18.1, 20.7, 20.8, 20.9, 21.0, 22.6, 26.0, 29.2–29.6, 31.8, 66.1, 66.5, 67.0, 67.1, 67.7, 69.3, 70.8, 70.9, 71.5, 71.9, 72.1, 72.2, 72.4, 74.7, 75.5, 78.5, 79.3, 99.5 (2C), 100.8, 101.8, 170.2, 170.4, 171.2, 171.8. HRESIMS: calcd for $C_{44}H_{74}O_{21}$ 938.4723; found, 961.4635 $[M+Na]^+$.

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Supplementary data

NMR spectra for nine compounds (**3**, **5**, **7**, **10**, **11**, **12**, **13**, **14**, and **15**) are provided in the Supplementary data that will be published in the electronic version of this journal. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication with CCDC No. 646236. Copies of the data can be obtained free of charge on application with the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.05.019.

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