Synthesis of a pentasaccharide derivative corresponding to a triterpenoid saponin isolated from *Spergularia ramosa*

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A highly convergent and regioselective glycosylation strategy has been employed for the synthesis of a hetero-pentasaccharide that corresponds to the major sugar chain of a saponin isolated from herbaceous plant, *Spergularia ramosa*.

Introduction

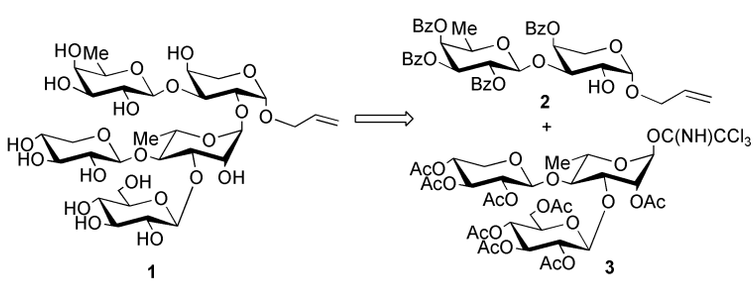
Saponins, the glycosides of steroids or triterpenes, are widely distributed in plants and animals.[1] Many studies reveal that saponins show excellent physiological and pharmacological activities, such as anti-cancer, anti-inflammatory, cardiovascular, and cytotoxic activities.[1,2] They also exhibit important roles in the cell-mediated immune response and in inter- and intracellular communication processes.[3] The oligosaccharides have a very important role in the bioactivity of saponins. For example, when the ester-linked tetrasaccharide was removed from julibrosides, its cytotoxicity dramatically decreased.[4] We also have demonstrated that a saponin analog, containing the same julibroside tetrasaccharide, shows mild activity against mouse leukaemia P388.[5]

Spergularia ramosa is a herbaceous plant from Perù.[6] The leaves of this species are used to feed sheep and, in the form of a decoction, it is used as a remedy for respiratory ailments, tuberculosis and rickets. Recent phytochemical investigation of the methanol extract from the aerial parts of *Spergularia ramosa* uncovered six new oleanene saponins, which possess gysogenin or quillaic acid as the aglycon. Interestingly, the oligosaccharide chains linked to C-28 of the aglycons are made up of five different saccharide residues as D-glucose, D-xylose, L-rhamnose, D-fucose and L-arabinose. Attracted by its complex structure and curious of the structure–activity relationship, we synthesized this highly branched oligosaccharide of *Spergularia ramosa* through a convergent regio- and stereoselective strategy.

Results and discussion

The retrosynthetic analysis of the target molecule **1** leads to the disaccharide acceptor **2** and trisaccharide trichloroacetimidate donor **3** (Scheme 1). The synthesis started with the preparation of β-(1⟶3)-linked disaccharide **2**. To this end, compound **4**[7] was acetylated with acetic anhydride in pyridine (⟶ **5**), followed by cleavage of acetonide in 90% aqueous trifluoroacetic acid (TFA) giving 3,4-diol **6** in 94% yield (Scheme 2). Direct condensation of **6** and fucopyranosyl donor **7**[8] in anhydrous CH_2Cl_2 in the presence of TMSOTf gave the undesired β-(1⟶4) disaccharide **8** in 65% yield. The selectivity did not change significantly when the reaction was carried out at −40 °C. The 1⟶4 linkage in **8** was confirmed by a downfield shift of H-3 (δ 5.47 ppm, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 3.5 Hz) in the ^{1}H NMR spectra of its benzoylated derivative, **9**. The attempted regioselective benzoylation of **6**, with benzoyl chloride in pyridine at −10 °C–0 °C, unexpectedly afforded **10**, as a major product (~70% yield). When **6** was treated with *tert*-butylchlorodimethylsilane and imidazole in pyridine, the 3-*O*-silylated **11** was obtained in 91% yield. Benzoylation of **11** with benzoyl chloride in pyridine (⟶ **12**), followed by desilylation in 90% TFA, gave **13** in an overall yield of 90%.[9] Glycosylation of **13** and **7** in anhydrous CH_2Cl_2 with TMSOTf as catalyst afforded β-(1⟶3)-linked disaccharide **14**. Deacetylation of **14** with acetyl chloride in CH_2Cl_2–MeOH[10] gave acceptor **2** in a total yield of 81% (from **13**).

In our previous synthesis of julibroside[5] and rhamnose-containing oligosaccharides,[11] we showed that regioselective glycosylation on 3-OH of rhamnose derivatives could be achieved by a two-step procedure, orthoester formation and rearrangement, using sugar bromides as donors. We also successfully synthesized L-arabinofuranosyl octamer using trichloroacetimidates as glycosyl donors and unprotected or partially protected arabinofuranosides as glycosyl acceptors.[12] We thus decided to undertake the direct glycosylation of trichloroacetimidate donor and rhamnose acceptor to produce the β-(1⟶3)-linked disaccharide in a one-pot reaction. Coupling of trichloroacetimidate **16** and 3,4-diol **15** in the presence of catalytic amounts of TMSOTf at −42 °C⟶0 °C gave desired product **17** in 63% yield. The presence of the β-(1⟶3) glycosyl



Scheme 1 Retrosynthetic analysis.

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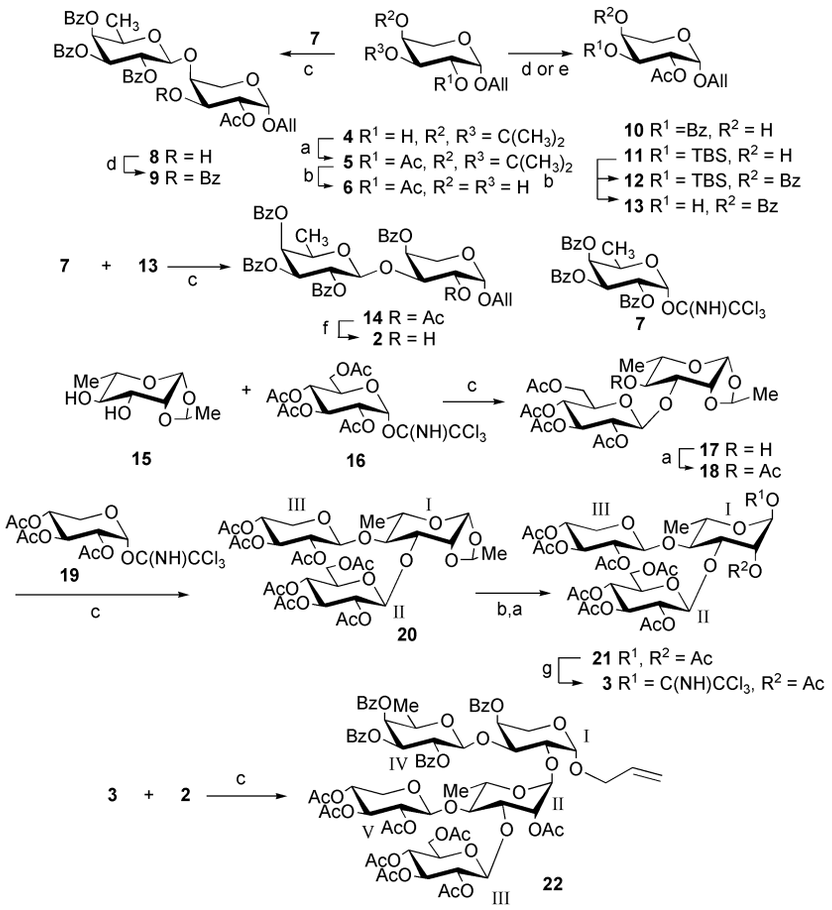

Scheme 2 *Reagents and conditions (yields)*: a) Ac_2O, Pyr (100% for **5**, 100% for **18**); b) 90% TFA (94% for **6**, 94% for **13**, 88% for **21**); c) TMSOTf, CH_2Cl_2 (65% for **8**, 87% for **14**, 63% for **17**, 81% for **20**, 83% for **22**); d) BzCl, Pyr (93% for **9**, 70% for **10**, 95% for **12**); e) TBSCl, Im, Pyr (91%); f) MeOH, AcCl (94%); g) NH_3, MeOH–THF (3 : 7), then CCl_3CN, DBU, CH_2Cl_2 (84% for two steps).

bond formation in **17** was supported by NMR analysis of the acetylated derivative **18**. The chemical shifts of H-4 (d 4.99 ppm) and H-1′ (d 4.77 ppm, *J* 8.0 Hz) in **18** confirmed 1⟶3 linkage in **17**. Condensation of **17** and **19** in CH_2Cl_2 at 0 °C, using TMSOTf as catalyst, generated **20** as a *R,S*-mixture in high yield.[13] Trisaccharide donor **3** was prepared in 73% overall yield through protection group manipulation[5] of **20**, *i.e.*, de-ethylidenation in 90% TFA, acetylation with acetic anhydride in pyridine (⟶ **21**), deacetylation of the anomeric carbon in ammonia saturated THF : MeOH (7 : 3) followed by trichloroacetimidate formation.[14] Finally, coupling of disaccharide acceptor **2** and trisaccharide donor **3** proceeded smoothly in anhydrous dichloromethane in the presence of TMSOTf completing the synthesis of pentasaccharide **22** in 83.4% isolated yield.

In conclusion, an efficient and convergent synthesis of the triterpenoid pentasaccharide was achieved in a regio- and stereoselective manner. Pentasaccharide **22**, having only acyl protecting groups, could be used for the total synthesis of saponin of *Spergularia ramosa*. This strategy also provides an entry to the preparation of oligosaccharides with other structures than those isolated from *Spergularia ramosa*.

Experimental

General methods

Optical rotations were determined at 20 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. Melting points were determined with a "Mel-Temp" apparatus. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY 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 using MALTI-TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. 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 silica gel column with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Solutions were concentrated at <60 °C under diminished pressure.

Allyl 2-*O*-acetyl-β-L-arabinopyranoside 6

Compound **4** (1.1 g, 4.78 mmol) was acetylated in pyridine (10 mL) with acetic anhydride (3 mL) at room temperature for 4 h, then co-evaporated with toluene 3 times (3 × 30 mL). The residue was dissolved in 90% TFA (20 mL), stirred at room temperature for 30 min and concentrated with the help of toluene *in vacuo*. Purification of the residue by silica gel column chromatography using petroleum ether–EtOAc 1 : 1 as eluent gave crystalline **6** (1.04 g, 94%); $[\alpha]_D^{20}$ +117 (*c* 5, $CHCl_3$); mp: 67–69 °C; ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3 H, CH_3CO), 3.94 (dd, 1 H, $J_{5a,5b}$ 12.6, $J_{5a,4}$ 1.8 Hz, H-5a), 3.91 (dd, 1 H, $J_{5b,4}$1.2 Hz, H-5b), 3.98–4.04 (m, 2 H, H-4, one proton of CH_2=CH–CH_2-), 4.08 (dd, 1 H, $J_{3,4}$ 3.5, $J_{3,2}$ 9.7 Hz, H-3), 4.17–4.20 (m, 1 H, one proton of CH_2=CH–CH_2-), 4.99–5.05 (m, 2 H, H-1, H-2), 5.19–5.35 (m, 2 H, CH_2=CH–CH_2-), 5.84–5.92 (m, 1 H, CH_2=CH–CH_2-) (Calc. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.53; H, 7.04%).

Allyl 2,3,4-tri-*O*-benzoyl-β-D-fucopyranosyl-(1⟶4)-2-*O*-acetyl-3-*O*-benzoyl-β-L-arabinopyranoside 9

To a solution of **7** (281 mg, 0.454 mmol) and **6** (100 mg, 0.431 mmol) in anhydrous CH_2Cl_2 (4 mL) was added TMSOTf (8.2 μL, 0.045 mmol) under a N_2 atmosphere at 0 °C. The

mixture was stirred at these conditions for 1.5 h, at the end of which time TLC (3 : 1 petroleum ether–EtOAc) indicated the completion of the reaction. The reaction mixture was neutralized with triethylamine, and concentrated. The residue was purified on a silica gel column using 3 : 1 petroleum ether–EtOAc as eluent to give syrupy **8**. To the above residue in CH_2Cl_2 (3 mL) was added pyridine (3 mL) and benzoyl chloride (100 μL). The mixture was stirred at room temperature for 4 h and flash column separation gave **9** as a syrup (60.5% for two steps); $[a]_D^{20}$ +168 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.20 (d, 3 H, *J* 6.4 Hz, H-6′), 1.89 (s, 3 H, C*H*$_3$CO), 3.92 (br q, 1 H, H-5′), 3.99–4.05 (m, 3 H, H-5a,5b and one proton of CH_2=CH–C*H*$_2$-), 4.18–4.23 (m, 1 H, one proton of CH_2=CH–C*H*$_2$-), 4.46 (br s, 1 H, H-4), 4.88 (d, 1 H, *J* 8.0 Hz, H-1′), 5.15 (d, 1 H, *J* 3.4 Hz, H-1), 5.16–5.33 (m, 3 H, H-2, C*H*$_2$=CH–CH$_2$-), 5.45 (dd, 1 H, *J* 3.5, 10.4 Hz, H-3′), 5.47 (dd, 1 H, *J* 3.5, 10.8 Hz, H-3), 5.62 (d, 1 H, *J* 3.5 Hz, H-4′), 5.76 (dd, 1 H, *J* 10.4, 8.0 Hz, H-2′), 5.82–5.93 (m, 1 H, CH_2=C*H*–CH_2-), 7.20–8.12 (m, 20 H, Ph) [Calc. for $C_{44}H_{42}NaO_{14}$ (M + Na)$^+$: 817.26. Found: *m*/*z*, 817.3 (M + Na)$^+$].

Allyl 2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-β-L-arabinopyranoside 11

To a solution of compound **6** (336 mg, 1.45 mmol) in pyridine (5 mL) at 0 °C was added *tert*-butyldimethylsilyl chloride (229 mg, 1.52 mmol) and imidazole (100 mg, 1.5 mmol). The mixture was stirred at room temperature for about 4 h and quenched by one drop of methanol, and then co-evaporated with toluene under diminished pressure to dryness. The residue was purified by column chromatography using 5 : 1 petroleum ether–EtOAc as eluent to give syrupy **11** (458 mg, 91.4%); $[a]_D^{20}$ +156 (*c* 3.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.12, 0.13 (2 s, 6 H, 2 Si(C*H*$_3$)$_2$), 0.90 (s, 9 H, C(C*H*$_3$)$_3$), 2.09 (s, 3 H, C*H*$_3$CO), 3.76 (dd, 1 H, $J_{5a,5b}$12.4, $J_{5a,4}$1.8 Hz, H-5a), 3.82 (dd, 1 H, $J_{5b,4}$1.4 Hz, H-5b), 3.84–3.87 (m, 1 H, H-4), 3.96–4.02 (m, 1 H, one proton of CH_2=CH–C*H*$_2$-), 4.11–4.20 (m, 2 H, H-3, one proton of CH_2=CH–C*H*$_2$-), 4.99 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 5.03 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.17–5.32 (m, 2 H, C*H*$_2$=CH–CH$_2$-), 5.84–5.91 (m, 1 H, CH_2=C*H*–CH_2-) (Calc. for $C_{16}H_{30}O_6Si$: C, 55.46; H, 8.73. Found: C, 55.73; H, 8.65%).

Allyl 2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-4-*O*-benzoyl-β-L-arabinopyranoside 12

To a solution of compound **11** (430 mg, 1.24 mmol) in pyridine (5 mL) was added benzoyl chloride (1.72 mmol, 0.2 mL) at 0 °C. The mixture was stirred at room temperature for 10 h, then co-evaporated with toluene under reduced pressure. Column chromatography (9 : 1 petroleum ether–EtOAc) of the residue gave **12** (531 mg, 95.2%) as a syrup; $[a]_D^{20}$ +158 (*c* 2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.20, 0.23 (2 s, 6 H, 2 Si(C*H*$_3$)$_2$), 0.90 (s, 9 H, C(C*H*$_3$)$_3$), 2.26 (s, 3 H, C*H*$_3$CO), 3.95 (dd, 1 H, $J_{5a,5b}$12.8, $J_{5a,4}$ 2.3 Hz, H-5a), 4.10–4.19 (m, 2 H, H-5b, one proton of CH_2=CH–C*H*$_2$-), 4.32–4.38 (m, 1 H, one proton of CH_2=CH–C*H*$_2$-), 4.44 (dd, 1 H, $J_{3,4}$3.6, $J_{3,2}$ 9.2 Hz, H-3), 5.25–5.29 (m, 2 H, H-1, H-2), 5.34–5.48 (m, 2 H, C*H*$_2$=CH–CH$_2$-), 5.51 (ddd, 1 H, H-4), 5.99–6.05 (m, 1 H, CH_2=C*H*–CH_2-), 7.56–8.23 (m, 5 H, Ph) (Calc. for $C_{23}H_{34}O_7Si$: C, 61.31; H, 7.61. Found: C, 61.55; H, 7.52%).

Allyl 2-*O*-acetyl-4-*O*-benzoyl-β-L-arabinopyranoside 13

A solution of **12** (466 mg, 1.04 mmol) in 90% aqueous TFA (4 mL) was stirred at room temperature for 2 h, then neutralized with saturated aqueous sodium bicarbonate and extracted with dichloromethane (3 × 15 mL). The organic phases were combined, dried over anhydrous sodium sulfate and concentrated. Purification of the residue on a silica gel column using 3 : 1 petroleum ether–EtOAc as eluent afforded **13** (330 mg, 94.3%) as white solid; $[a]_D^{20}$ +212 (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3 H, C*H*$_3$CO), 3.88 (dd, 1 H, $J_{5a,5b}$13.1, $J_{5a,4}$1.9 Hz, H-5a), 3.98–4.06 (m, 2 H, $J_{5b,4}$1.2 Hz, H-5b, one proton of CH_2=CH–C*H*$_2$-), 4.18–4.24 (m, 1 H, one proton of CH_2=CH–C*H*$_2$-), 4.33 (dd, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.13 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.19 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 5.21–5.37 (m, 2 H, C*H*$_2$=CH–CH$_2$-), 5.44 (ddd, 1 H, H-4), 5.86–5.93 (m, 1 H, CH_2=C*H*–CH_2-), 7.44–8.11 (m, 5 H, Ph) (Calc. for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99. Found: C, 60.79; H, 6.05%).

Allyl 2,3,4-tri-*O*-benzoyl-β-D-fucopyranosyl-(1⟶3)-2-*O*-acetyl-4-*O*-benzoyl-β-L-arabinopyranoside 14

To a solution of **13** (300 mg, 0.893 mmol) and **7** (582 mg, 0.937 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TMSOTf (18 μL, 0.10 mmol) under a N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 1.5 h, at the end of which time TLC (3 : 1 petroleum ether–EtOAc) indicated the completion of the reaction. The reaction mixture was neutralized with triethylamine, and concentrated. The residue was purified on a silica gel column using 3 : 1 petroleum ether–EtOAc as eluent to give syrupy **14** (615 mg, 86.7%); $[a]_D^{20}$ +195 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.18 (d, $J_{6',5'}$ 6.4 Hz, H-6′), 1.60 (s, 3 H, C*H*$_3$CO), 3.89–3.96 (m, 2 H, H-5a, one proton of CH_2=CH–C*H*$_2$-), 3.98–4.18 (m, 2 H, H-5b, H-5′), 4.13–4.18 (m, 1 H, one proton of CH_2=CH–C*H*$_2$-), 4.35 (dd, 1 H, $J_{3,4}$ 3.7, $J_{3,2}$ 9.9 Hz, H-3), 4.96 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1′), 5.06 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.17–5.28 (m, 3 H, H-2, C*H*$_2$=CH–CH$_2$-), 5.50 (dd, 1 H, $J_{3,4}$ 3.4, $J_{3,2}$ 10.4 Hz, H-3′), 5.57 (ddd, 1 H, H-4), 5.61–5.67 (m, 2 H, H-2′, H-4′), 5.76–5.86 (m, 1 H, CH_2=C*H*–CH_2-), 7.21–8.14 (m, 20 H, Ph) (Calc. for $C_{44}H_{42}O_{14}$: C, 66.49; H, 5.33. Found: C, 66.56; H, 5.41%).

Allyl 2,3,4-tri-*O*-benzoyl-β-D-fucopyranosyl-(1⟶3)-4-*O*-benzoyl-β-L-arabinopyranoside 2

To a solution of **14** (580 mg, 0.730 mmol) in MeOH–CH_2Cl_2 (v/v 1 : 1, 30 mL) was added acetyl chloride (2 mL) in 10 min at 0 °C. The reaction mixture was stirred at room temperature overnight, then neutralized with saturated aqueous $NaHCO_3$, extracted with dichloromethane (2 × 25 mL). The organic phases were combined, dried with anhydrous Na_2SO_4, concentrated under reduced pressure to dryness, then passed through a silica gel column with 2 : 1 petroleum ether–EtOAc as eluent to afford the disaccharide acceptor **2** (515 mg, 93.8%) as a syrup; $[a]_D^{20}$ +180 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.20 (d, 3 H, $J_{6,5}$ 6.4 Hz, H-6′), 3.89 (dd, 1 H, $J_{5a,5b}$11.4, $J_{5a,4}$ 2.6 Hz, H-5a), 3.94–4.06 (m, 3 H, H-2, H-5b, one proton of CH_2=CH–C*H*$_2$-), 4.09–4.15 (m, 1 H, H-5′), 4.16–4.24 (m, 2 H, H-3, one proton of CH_2=CH–C*H*$_2$-), 4.92 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.10 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1′), 5.20–5.32 (m, 2 H, C*H*$_2$=CH–CH$_2$-), 5.48 (br s, 1 H, H-4), 5.52 (dd, 1 H, $J_{3,4}$ 3.4, $J_{3,2}$ 10.4 Hz, H-3′), 5.63–5.69 (m, 2 H, H-2′, H-4′), 5.84–5.94 (m, 1 H, CH_2=C*H*–CH_2-), 7.21–8.06 (m, 20 H, Ph) (Calc. for $C_{42}H_{40}O_{13}$: C, 67.01; H, 5.36. Found: C, 66.92; H, 5.40%).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶3)-1,2-*O*-ethylidene-β-L-rhamnopyranose 17

The reaction mixture of **15** (270 mg, 1.42 mmol) and **16** (698 mg, 1.42 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL). TMSOTf (7.5 μL, 0.04 mmol) was added dropwise at −42 °C under N_2 protection. The mixture was stirred at this temperature for 2 h, then stirred at 0 °C for 16 h, neutralized with Et_3N and concentrated under reduced pressure to dryness. Purification of the residue by column chromatography (2 : 1 petroleum ether–EtOAc) gave **17** (*R*,*S* mixture, 465 mg, 63%) as a syrup.[11]

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶3)-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1⟶4)]-1,2-*O*-ethylidene-β-L-rhamnopyranose 20

Compound **17** (584 mg, 1.12 mmol) and **19** (481 mg, 1.14 mmol) were pre-dried in one flask under vacuum at 60 °C for

3 h. The mixture was then dissolved in anhydrous CH_2Cl_2 (5 mL). To the solution was added TMSOTf (15 μL, 0.083 mmol) under a N_2 atmosphere at 0 °C. The reaction mixture was stirred at this temperature for 1.5 h, then neutralized with triethylamine, concentrated under reduced pressure and purified on a silica gel column with 2 : 1 petroleum ether–EtOAc as eluent to give a syrupy *R,S*-mixture of **20** (710 mg, 81.4%); ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 1.2 H, $J_{6,5}$ 6.0 Hz, *S*–H-6^{I}), 1.30 (d, 1.8 H, $J_{6,5}$ 6.0 Hz, *R*–H-6^{I}), 1.36 (d, 1.2 H, *S*-CHC*H*$_3$), 1.51 (d, 1.8 H, *R*-CHC*H*$_3$), 2.01 (br s, 3 H, C*H*$_3$CO), 2.04 (br s, 6 H, 2 C*H*$_3$CO), 2.05 (br s, 3 H, C*H*$_3$CO), 2.08 (s, 1.8 H, C*H*$_3$CO), 2.09 (s, 1.2 H, C*H*$_3$CO), 2.15 (s, 1.2 H, C*H*$_3$CO), 2.17 (s, 1.8 H, C*H*$_3$CO), 2.18 (br s, 3 H, C*H*$_3$CO), 3.31–3.43 (m, 1.8 H, H-5^{I}, *S*-H-5a^{III} and *S*-H-5b^{III}), 3.69 (m, 1 H, H-5^{II}), 3.80–3.94 (m, 1.6 H, *S*-H-3^{I}, *R*-H-5a^{III} and *R*-H-5b^{III}), 4.06–4.13 (m, 1.2 H, *R*-H-3^{I} and H-4^{I}), 4.15–4.24 (m, 3 H, *R*-H-2^{I}, *S*-H-4^{I}, H-6a^{II}, H-6b^{II}), 4.31 (br t, 0.4 H, *S*-H-2^{I}), 4.77 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1^{III}), 4.83–4.93 (m, 3.2 H, *R*-H-2^{II}, *R*-H-2^{III} and H-4^{II}, H-4^{III}), 5.06–5.14 (m, 3 H, H-1^{II}, *S*-H-2^{II}, *S*-H-2^{III}, *R*-H-3^{II} and *R*-H-3^{III}), 5.15–5.19 (m, 1.4 H, *R*-H-1^{I}, *S*-H-3^{III} and *S*-H-3^{II}), 5.24–5.28 (m, 1 H, *S*-H-1^{I}, *R*–C*H*CH$_3$), 5.62 (q, 0.4 H, *S*–C*H*CH$_3$) [MALDI TOF-MS calc. for $C_{33}H_{46}O_{21}$: 778.25; Found: 801.2 [M + Na]$^+$] (Calc. for $C_{33}H_{46}O_{21}\cdot H_2O$: C, 49.70; H, 6.02. Found: C, 49.48; H, 6.10%).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶3)-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1⟶4)]-1,2-di-*O*-acetyl-α-L-rhamnopyranose 21

A solution of compound **20** (662 mg, 0.85 mmol) in aqueous 90% TFA (5 mL) was stirred at room temperature for 0.5 h, then co-evaporated with toluene under diminished pressure to dryness. The above product was then dissolved in pyridine (4 mL). To the above mixture was added acetic anhydride (3 mL). The reaction mixture was stirred at room temperature overnight, then evaporated under reduced pressure to dryness. The residue was purified by column chromatography using 1 : 1 petroleum ether–EtOAc to afford **21** (623 mg, 87.6% for two steps) as a white solid; $[\alpha]_D^{20}$ +65 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3 H, $J_{6,5}$ 6.1 Hz, H-6^{I}), 2.01, 2.03, 2.04, 2.06, 2.08, 2.13, 2.15, 2.16, 2.19 (9 s, 9 × 3 H, 9 C*H*$_3$CO), 3.43 (dd, 1 H, $J_{5a,5b}$12.0, $J_{5a,4}$7.8 Hz, H-5a^{III}), 3.65–3.74 (m, 2 H, H-5^{I}, H-5^{II}), 3.82 (t, 1 H, $J_{4,3} = J_{4,5}$ 9.3 Hz, H-4^{I}), 4.05 (dd, 1 H, $J_{4,3}$ 3.6 Hz, H-3^{I}), 4.10–4.17 (m, 2 H, H-5b^{III}, H-6a^{II}), 4.20 (dd, 1 H, $J_{6a,6b}$12.3, $J_{6b,5}$ 2.2 Hz, H-6b^{II}), 4.73 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{III}), 4.83–4.93 (m, 3 H, H-1^{II}, H-4^{II}, H-4^{III}), 4.98–5.19 (m, 5 H, H-2^{I}, H-2^{II}, H-2^{III}, H-3^{II}, H-3^{III}), 5.94 (d, 1 H, $J_{1,2}$1.8 Hz, H-1^{I}) (Calc. for $C_{35}H_{48}O_{23}$: C, 50.24; H, 5.78. Found: C, 50.41; H, 5.83%).

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶3)-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1⟶4)]-2-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate 3

Into a solution of **21** (583 mg, 0.697 mmol) in MeOH–THF (v/v 3 : 7, 10 mL) ammonia was bubbled at room temperature for 15 min, and then stirred at this temperature for 1.5 h, at the end of which time TLC indicated the completion of the reaction. The reaction mixture was evaporated under reduced pressure to dryness. The above residue was then dissolved in anhydrous CH_2Cl_2 (6 mL), and trichloroacetonitrile (0.21 mL, 2.1 mmol) and DBU (0.05 mL, 0.33 mmol) were added sequentially. The reaction mixture was stirred at room temperature for 2 h, and then concentrated. Purification of the residue on a silica gel column with 3 : 2 petroleum ether–EtOAc as eluent gave the trisaccharide donor **3** (547 mg, 83.7% for two steps) as a foamy solid; $[\alpha]_D^{20}$ −69 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.32 (d, 3 H, $J_{6,5}$ 5.6 Hz, H-6^{I}), 2.00, 2.02, 2.03, 2.06, 2.08, 2.14, 2.15, 2.19 (8 s, 8 × 3 H, 8 C*H*$_3$CO), 3.43 (dd, 1 H, $J_{5a,5b}$12.0, $J_{5a,4}$ 7.7 Hz, H-5a^{III}), 3.67 (m, 1 H, H-5^{II}), 3.82–3.87 (m, 2 H, H-5^{I}, H-5b^{III}), 4.07–4.15 (m, 4 H, H-3^{I}, H-4^{I}, H-6a^{II}, H-6b^{II}), 4.73 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{III}), 4.83–4.94 (m, 3 H, H-1^{II}, H-4^{II}, H-4^{III}), 5.02 (dd, 1 H, $J_{2,1}$ 7.8, $J_{2,3}$ 9.3 Hz, H-2$^{II/III}$), 5.08–5.20 (m, 3 H, H-2$^{III/II}$, H-3^{II}, H-3^{III}), 5.35 (dd, 1 H, H-2^{I}), 6.13 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^{I}), 8.71 (s, 1 H, =N*H*) (Calc. for $C_{35}H_{46}Cl_3NO_{22}$: C, 44.76; H, 4.94. Found: C, 44.48; H, 4.85%).

Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶3)-[2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1⟶4)]-2-*O*-acetyl-α-L-rhamnopyranosyl-(1⟶2)-[2,3,4-tri-*O*-benzoyl-β-D-fucopyranosyl-(1⟶3)]-4-*O*-benzoyl-β-L-arabinopyranoside 22

To a solution of **2** (400 mg, 0.532 mmol) and **3** (524 mg, 0.558 mmol) in anhydrous dichloromethane (5 mL) was added Me_3SiOTf (15 μL, 0.083 mmol) at 0 °C with a N_2 atmosphere. The reaction mixture was stirred at this condition for 1.5 h, at the end of which time TLC (1 : 1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with triethylamine and then concentrated. The residue was purified on a silica gel column using 1 : 1 petroleum ether–EtOAc as eluent to give the pentasaccharide **22** (678 mg, 83.4%) as a syrup; $[\alpha]_D^{20}$ +42 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.18 (d, 3 H, $J_{6,5}$ 6.8 Hz, H-6^{IV}), 1.20 (d, 3 H, $J_{6,5}$ 6.2 Hz, H-6^{II}), 2.00, 2.01, 2.02, 2.04, 2.05, 2.08, 2.12, 2.19 (8 s, 8 × 3 H, 8 C*H*$_3$CO), 3.37 (dd, 1 H, $J_{5a,5b}$ 11.8, $J_{5a,4}$ 9.1 Hz, H-5a^{V}), 3.68–3.88 (m, 5 H, H-4^{II}, H-5a^{I}, H-5^{II}, H-5^{III}, one proton of CH_2=CH–C*H*$_2$-), 3.91–4.02 (m, 3 H, H-2^{I}, H-3^{II}, H-5b^{I}), 4.05–4.17 (m, 4 H, H-5^{IV}, H-5b^{V}, H-6a^{III}, one proton of CH_2=CH–C*H*$_2$-), 4.27 (dd, 1 H, $J_{6b,6a}$11.4, $J_{6b,5}$ 5.1 Hz, H-6b^{III}), 4.48 (dd, 1 H, $J_{3,2}$ 3.6, $J_{3,4}$ 9.1 Hz, H-3^{I}), 4.71–4.84 (m, 4 H, H-1^{I}, H-1^{III}, H-1^{V}, H-2^{V}), 4.86–4.97 (m, 2 H, H-1^{II}, H-4^{V}), 4.99–5.23 (m, 7 H, H-1^{IV}, H-3^{III}, H-2^{III}, H-4^{III}, H-3^{V}, C*H*$_2$=CH–CH$_2$-), 5.32 (dd, 1 H, *J* 1.9, 3.8 Hz, H-2^{II}), 5.49 (br t, 1 H, H-4^{I}), 5.59 (dd, 1 H, $J_{3,2}$10.4, $J_{3,4}$ 3.5 Hz, H-3^{IV}), 5.60–5.72 (m, 3 H, H-2^{IV}, H-4^{IV}, CH$_2$=C*H*–CH$_2$-), 7.23–8.13 (m, 20 H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 16.06 (*C*-6^{IV}), 17.82 (*C*-6^{I}), 20.58, 20.67, 20.80, 20.97, 21.04 (8 C, *C*H$_3$CO, some overlapped), 60.85 (*C*-5^{I}), 61.12 (*C*-6^{III}), 61.75 (*C*-5^{V}), 66.80 (*C*-5^{III}), 67.89 (*C*-4^{III}), 68.91 (CH$_2$=CH-*C*H$_2$-), 68.96 (*C*-4^{V}), 69.38 (*C*-4^{I}), 70.05 (*C*-5^{IV}), 71.00 (*C*-2^{IV}), 71.14 (2 C, *C*-2^{II}, *C*-4^{IV}), 71.24 (*C*-3^{V}), 71.53 (*C*-5^{II}), 71.68 (*C*-2^{V}), 71.96 (*C*-3^{IV}), 72.07 (*C*-2^{III}), 72.17 (*C*-3^{I}), 73.12 (*C*-3^{III}), 73.85 (*C*-4^{II}), 74.65 (*C*-2^{I}), 78.86 (*C*-3^{II}), 97.10 (*C*-1^{I}), 98.04 (*C*-1^{II}), 98.67 (*C*-1^{V}), 99.38 (*C*-1^{IV}), 100.12 (*C*-1^{III}), 118.18 (*C*H$_2$ =CH–CH$_2$-), 132.90 (CH$_2$ =*C*H–CH$_2$-), 165.28, 165.39, 165.98, 166.01 (4 C, Ph*C*O), 169.19, 169.36, 169.81, 169.90, 169.93, 170.02, 170.13, 170.58 (8 C, CH$_3$*C*O) [Calc. for $C_{75}H_{84}O_{34}$: 1528.48; Found: 1551.5 [M + Na]$^+$ and 1567.5 [M + K]$^+$] (Calc. for $C_{75}H_{84}O_{34}$: C, 58.90; H, 5.54. Found: C, 58.99; H, 5.47%).

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