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.2,3 They also exhibit important roles in the cell-mediated immune response and in inter- and intracellular communication processes.4 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 julibrose tetrasaccharide, shows mild activity against mouse leukaemia P388.5

Spergularia ramosa is a herbaceous plant from Peru.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 oleane 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 β-glucose, β-xyllose, 1-rhamnose, β-fucose and 1-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 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 in anhydrous CH2Cl2 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, J2,3 10.8 Hz, J3,4 3.5 Hz) in the 1H NMR spectra of its benzoylated derivative, 9. The attempted regio-selective benzylation of 6, with benzyl chloride in pyridine at −10 °C to −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. Benzylation of 11 with benzyl chloride in pyridine (→ 12), followed by desilylation in 90% TFA, gave 13 in an overall yield of 90%. Glycosylation of 13 and 7 in anhydrous CH2Cl2 with TMSOTf as catalyst afforded β-(1→3)-linked disaccharide 14. Deacetylation of 14 with acetyl chloride in CH2Cl2–MeOH10 gave acceptor 2 in a total yield of 81% (from 13).

In our previous synthesis of julibrose4 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 1-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 to −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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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 CH2Cl2 at 0 °C, using TMSOTf as catalyst, generated 20 as a R,S-mixture in high yield. Trisaccharide donor 3 was prepared in 73% overall yield through protection group manipulation 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. 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. 1H NMR, 13C NMR and 1H-1H, 1H-13C COSY spectra were recorded with Bruker ARX 400 spectrometers for solutions in CDCl3. Chemical shifts are given in ppm downfield from internal Me4Si. 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 HF254. Chemical shifts (8.2 µL, 0.045 mmol) in CDCl3, DBU, CH2Cl2 (84% for two steps).

**Scheme 2** Reagents and conditions (yields): a) Ac₂O, Pyr (100% for 5, 100% for 18); b) 90% TFA (94% for 6, 94% for 13, 88% for 21); c) TMSOTf, CH3Cl, 65% for 8, 87% for 14, 63% for 17, 81% for 20, 83% for 22; d) BrCl, Pyr (93% for 9, 70% for 10, 95% for 12); e) TBSCI, Im, Pyr (91%); f) MeOH, AcCl (94%); g) NH3 in high yield.

**Allyl 2-O-acetyl-β-D-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%); [α]25/D +117 (c 5, CHCl3); mp: 67–69 °C; 1H NMR (CDCl3): δ 2.13 (s, 3 H, CH3CO), 3.94 (dd, 1 H, J5a,5b 12.6, J5a,5b 1.8 Hz, H-5a), 3.91 (dd, 1 H, J5b,4 1.2 Hz, H-5b), 3.98–4.04 (m, 2 H, CH–C), 4.08 (dd, 1 H, J3a,3b 3.5, 3.5, 9.7 Hz, H-3), 4.17–4.20 (m, 1 H, one proton of CH2=C–CH2-), 4.99–5.05 (m, 2 H, H-1, H-2), 5.19–5.35 (m, 2 H, CH2=C–CH2-), 5.84–5.92 (m, 1 H, CH2=C–CH2-)(Calc. for C10H13O2C: C, 51.72; H, 6.94. Found: C, 51.53; H, 7.04%).
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

To the solution of compound 6 (336 mg, 1.45 mmol) in pyridine (5 mL) at 0 °C was added tert-butylmethylsilylethyl-β-1-arabinopyranoside 11

To a solution of compound 11 (458 mg, 91.4%); [α]20D +156 (c 3.8, CHCl3); 1H NMR (CDCl3); δ 0.12, 0.13 (2, s, 6 H, 2 Si(CH3)2); 0.90 (s, 9 H, C(CH3)3); 2.09 (s, 3 H, CH3COO); 3.76 (dd, 1 H, Jα,α 12.4, Jα,β 1.8 Hz, H-5α); 3.82 (dd, 1 H, Jα,α 14.1 Hz, H-5β); 3.84–3.87 (m, 1 H, H-1); 3.96–4.02 (m, one proton of CH2–O–CH2–, 2.54) (1 H, 1H, J3.4 Hz, H-1, 5.15 (dd, 1 H, 1H, J3.4 Hz, H-1), 1.8–2.0 Hz, 13.5 H, C, CH–CH–CH2–, H-2), 5.26–5.32 (m, 2 H, CH2–CH–CH2–), 5.84–5.91 (m, 1 H, CH–CH–CH2–) (Calc. for C15H24O6Si: C, 55.46; H, 8.73. Found: C, 55.7; H, 8.65%).

Allyl 2-O-acetyl-3-O-tert-butylmethylsilylethyl-β-1-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; [α]20D +212 (c 1.3, CHCl3); 1H NMR (CDCl3); δ 2.16 (s, 3 H, CH3COO), 3.88 (dd, 1 H, Jα,β 13.1, Jβ,γ 4.9 Hz, H-5α), 3.98–4.06 (m, 2 H, Jα,β 1.2 Hz, H-5β, one proton of CH2–CH–CH2–, 4.18–4.24 (m, 1 H, one proton of CH2–CH–CH2–), 4.33 (dd, 1 H, Jα,α 3.7 Hz, H-3), 5.13 (d, 1 H, J3.4 3.6 Hz, H-2); 0.52 (d, 1 H, J1H, J3.4 Hz, 8.0 Hz, H-1, 1.34 (d, 1 H, J1H, J3.4 Hz, H-1), 5.44 (dd, 1 H, JH–H 4.8 Hz, H-2), 5.86–5.93 (m, 1 H, CH–CH–CH2–, 7.44–8.11 (m, 5 H, Ph) (Calc. for C16H20O3C: 60.71; H, 5.99. Found: C, 60.79; H, 6.05%).

Allyl 2,3,4,tri-O-benzoyl-β-1-fucopyranosyl-(1→3)-2-O-acetyl-4-O-benzoyl-β-1-arabinopyranoside 14

To a solution of 13 (300 mg, 0.893 mmol) and 7 (582 mg, 0.937 mmol) in anhydrous CH2Cl2 (5 mL) was added TMSOTf (18 µL, 0.10 mmol) under a N2 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

To a solution of 14 (515 mg, 93.8%) as a syrup: [α]20D +180 (c 1, CHCl3); 1H NMR (CDCl3); δ 1.20 (d, 3 H, J3.4 6.4 Hz, H-6'), 3.89 (dd, 1 H, Jα,α 11.4, Jα,β 2.6 Hz, H-5a), 3.94–4.06 (m, 3 H, H-2, H-5, one proton of CH2–CH–CH2–, 4.09–4.15 (m, 1 H, H-5'), 4.16–4.24 (m, 2 H, H-3, one proton of CH2–CH–CH2–), 4.92 (d, 1 H, Jα,α 3.4 Hz, H-2), 5.10 (d, 1 H, J3.4 3.7 Hz, H-3), 5.40 (dd, 1 H, J1H, J3.4 Hz, 10.4 Hz, H-5'), 5.57 (dd, 1 H, J1H, H-4'), 5.61–5.67 (m, 2 H, H-2', H-4'), 7.21–7.24 (m, 20 H, Ph) (Calc. for C25H24O16Si: C, 66.49; H, 5.33. Found: C, 66.56; H, 5.41%).

Allyl 2,3,4,tri-O-benzoyl-β-1-fucopyranosyl-(1→3)-4-O-benzoyl-β-1-arabinopyranoside 2
3 h. The mixture was then dissolved in anhydrous CHCl₃ (5 mL). To the solution was added TMSOTf (15 µL, 0.083 mmol) under a N₂ 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 syrup. R-s.mixture of 2 (710 mg, 81.4%); 1H NMR (CDCl₃): δ 1.29 (d, 3 H, J = 6.0 Hz, H-6), 1.36 (d, 3 H, H-2), 1.51 (d, 1 H, H-3), 2.01 (br s, 3 H, CH₂O), 2.04 (s, 6 H, 2 CH₂), 2.09 (s, 1.2 H, CH₂O), 2.17 (s, 1.2 H, CH₂O), 2.20 (br s, 3 H, CH₂O), 3.31–3.43 (m, 1.8 H, H-5, H-6), 5.38–5.39 (m, 1.6 H, H-3, H-4), 5.59 (m, 1.6 H, H-5, H-6) ppm. 2078 J. Chem. Soc., Perkin Trans. 1, 2002, 2075–2079

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Acknowledgements

References


9 We also tried the selective benzoylation of allyl 3-O-benzoyl-β-L-arabinopyranoside and no good regioselectivity between 2-OH and 4-OH was given.


13 R,S configuration may alternative in our assignments.