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→4)-α-L-rhamnopyranosyl (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. From the stem bark of Cleistopholis glauca and the leaves of Cleistopholis patens (Annonaceae), a number of partially acetylated oligorhamnoside derivatives were isolated. Among these compounds, cleistetroside-2 [dodecanyl 4-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-acetyl-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-4-O-acetyl-α-L-rhamnopyranosyl] was found to show significant in vitro antibacterial activity against Gram-positive bacteria. For investigation of the oligosaccharide’s structure–bioactivity relationships in respect to antibacterial activities, we have prepared a series of L-rhamnose oligosaccharides. 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-rhamnopyranose 1 was converted into dodecanyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (2) by a method similar to that of reported. Similarly, compound 1 was transformed into isopropyl 2,3,4-tri-O-acetyl-1-thio-α-L-rhamnopyranoside (3) under modified Helferich reaction conditions. Deacetylation of 3 with NaOMe in methanol gave the acceptor, isopropyl 1-thio-α-L-rhamnopyranoside (4). 2,3-O-Isopropylidenation of 4 with Me3C(OMe)2 in the presence of TsOH in acetone, and acetylation with Ac2O 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 TBSCI and imidazol in DMF at 50 °C to give the building block, isopropyl 4-O-acetyl-2,3-di-O-tert-butylmethyldisilyl-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.
according to a published procedure. 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 CH2Cl2 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. Side reactions, that is, thio-group transfer and sugar ring contraction, were not observed in this case. 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-2A) and 5.08 ppm (t, 1H, J 9.8 Hz, H-4A) in the corresponding NMR spectra of 10 confirmed the (1→3)-linkage in its precursor 9. Convergently, coupling of building blocks 7 and 8 using NIS-TMSOTf as catalysts in dry CH2Cl2 gave (1→4)-linked disaccharide 11, which was subjected to desilylation with TBAF 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 of 10 and 12 was carried out smoothly using NIS and TMSOTf as catalysts in dry CH2Cl2 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-2C, which supports a newly formed (1→3)-linkage in 13. Furthermore, H-1C (δ 5.40 ppm, J <1.0 Hz), C-1C (δ 9.94 ppm), and 1J_C,H (167 Hz) of 13.
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. The preliminary results were summarized in Table 2.

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. Experimental

1.1. General methods

Optical rotations were determined at 25 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. 1H NMR, 13C NMR and COSY, and HMBC 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 with Q-TOF mass spectrometer using the ESI technique to introduce the sample. Thin-layer chromatography (TLC) was performed on silica gel HF254 with detection by charring with 30% (v/v) H₂SO₄ 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 CH₂Cl₂ (160 mL) was added BF₃·Et₂O (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 CH₂Cl₂ (100 mL), washed with water (3 × 100 mL), neutralized by satd aq NaHCO₃, and washed with brine. The organic layers were combined, dried, and concentrated to give a residue. Purification

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Escherichia coli clinical strains 25922</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Klebsiella pneumoniae ATCC 46117</td>
<td>16</td>
</tr>
<tr>
<td>Klebsiella pneumoniae clinical strains 46117</td>
<td>16</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 10211</td>
<td>32</td>
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<tr>
<td>Pseudomonas aeruginosa clinical strains 10211</td>
<td>32</td>
</tr>
<tr>
<td>Staphylococcus ATCC 2923</td>
<td>8</td>
</tr>
<tr>
<td>Staphylococcus clinical isolates 25923</td>
<td>8</td>
</tr>
</tbody>
</table>
of the residue by column chromatography on silica gel (3 (9.1 g, 16.4 mmol) in AcOH (80 mL) and H2O (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 TBSCI (5.5 g, 36.2 mmol) were added at 0 °C. The mixture was then stirred at 55 °C for 12 h, diluted with water (200 mL) and extracted with EtOAc (3 × 200 mL). The organic phase was dried over anhyd Na2SO4 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: [α]D 25° = -50 (c 1, CHCl3); 1H NMR (400 MHz, CDCl3): δ 5.12 d (1H, J 7.2 Hz, H-5B), 4.24 s (9H, OCH3), 2.73–2.88 (m, 2H, H-3A/H-3B), 2.67–2.71 (m, 1H, H-5A/H-5B/H-4).
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 NH₂Cl. The water layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhyd Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel (1:1 petroleum ether–EtOAc) to give 12 (3.25 g, 70%) as an amorphous solid: [α]D¹⁰⁰ –72 (c 1.3, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 5.40 (d, 1H, J 1.3 Hz, H-1β), 4.95 (s, 1H, H-1α), 4.84 (s, 1H, J 9.7 Hz, H-4β), 4.17 (dd, 1H, J 5.6 Hz, H-3α), 3.96 (s, 1H, H-2α), 3.82–3.78 (m, 2H, H-5β, H-2β), 1.38–1.20 (m, 14H), 0.87 (t, 3H, J 6.4 Hz, 3H-6), 0.87 (t, 3H, J 6.4 Hz, 3H-7), 0.87 (t, 3H, J 6.4 Hz, 3H-8). ¹³C NMR (100 MHz): δ 134.0, 173.4, 174.9, 27.3, 31.6, 35.9, 63.6, 67.0, 67.7, 70.9, 71.6, 72.5, 74.0, 74.2, 75.4, 75.9, 76.2, 77.8, 78.3, 86.4, 97.6, 97.9, 98.9, 99.4, 109.4, 109.6, 169.9, 169.9, 170.0, 170.3. HREIMS: calcd for C₂₉H₅₂O₁₀Si: 510.3549; found, 510.3547 [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 Ac₂O (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: [α]D²⁰ –60 (c 0.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 5.24 (d, 1H, J 1.6 Hz, H-1α), 5.22 (dd, 1H, J 1.6, 3.1 Hz, H-2α), 5.07 (t, 1H, J 6.4 Hz, H-4δ), 5.05 (s, 1H, H-3β), 5.02 (t, 1H, J 10.0 Hz, H-4β), 4.92 (s, 1H, H-1α), 4.90 (dd, 1H, J 1.7, 3.2 Hz, H-2β), 4.86 (dd, 1H, J 1.7 Hz, H-1β), 4.78 (dd, 1H, J 8.1, 10.0 Hz, H-4δ), 4.15 (dd, 1H, J 5.7, 7.1 Hz, H-4α), 4.07 (dd, 1H, J 5.5 Hz, H-2α), 4.05 (dd, 1H, J 5.5, 7.1 Hz, H-3α), 4.00–3.96 (m, 3H, H-2β, H-3β, H-4δ), 3.89–3.86 (m, 1H, H-5β), 3.79–3.75 (m, 1H, H-5α), 3.67–3.59 (m, 3H, H-5β, H-5δ, OCH₃H₂β), 3.45 (dd, 1H, J 7.3, 9.9 Hz, H-3δ), 3.42–3.39 (m, 1H, OCH₃H₂β), 2.16, 2.12, 2.09, 2.08, 2.07 (5s, 5×3H, 5×3H, 5×3H, 5×3H), 1.57–1.55 (m, 2H, OCH₃H₂β), 1.51, 1.50, 1.30, 1.29 (4s, 4×3H, 2(CH₃)₂C), 1.26 (d, J 6.4 Hz, H-6α), 1.24 (s, 18H, 9CH₃), 1.17, 1.16, 1.09 (3d, 3×3H, J 6.4 Hz, H-6β, H-6δ, H-6δ), 0.85 (t, 3H, J 7.0 Hz, CH₃). ESIMS: calcd for C₆₅H₁₁₀O₃₅Si: 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)-2,3-di-O-isopropylidene-α-L-rhamnopyranoside (15)

To a solution of compound 13 (500 mg, 0.49 mmol) in AcOH (8 mL) and H₂O (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 gumy solid: [α]D²⁰ –64 (c 1, MeOH), [lit. [α]D²⁰ –59.5 (c 0.4, MeOH), [lit. [α]D²⁰ –63.9 (c 0.4, MeOH)]. ¹H NMR (400 MHz, CDCl₃): 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-5 D), 3.67–3.60 (m, 3H, H-5 A, H-5 D, OCH a H 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 2C H 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 B), 1.10 (d, 3H, J 6.2 Hz, H-6 D), 0.88 (t, 3H, J 7.0 Hz, CH 3). 13C 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: cacld for C44H74O21 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.

References