Short synthesis of the common trisaccharide core of kankanose and kankanoside isolated from *Cistanche tubulosa*

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**Abstract**

A short synthetic approach was developed for the synthesis of a common trisaccharide core found in kankanose, kankanoside F, H₁, H₂, and I isolated from the medicinally active plant *Cistanche tubulosa*. All glycosylations were carried out under nonmetallic reaction conditions. Yields were very good in all intermediate steps.

**Introduction**

*Cistanche tubulosa* (*C. tubulosa*), an *Orobanchaceae* parasitic plant found in Africa, Asia and Arabia, has been traditionally used as folk medicine and tonic for the treatment of blood-circulation-related disorders, impotence, sterility and body weakness [1-3]. A significant number of bioactive compounds have been isolated from *C. tubulosa* and have shown promising medicinal activity such as hepatoprotective and vasorelaxant activities [4-6]. Most of the compounds isolated from *C. tubulosa* and related species are phenylethyl oligosaccharides, iridoids, terpenes and lignans [4-7]. Recently, Yoshikawa et al. isolated and characterized a significant number of phenylethyl oligosaccharides, which include kankanose, kankanoside F, H₁, H₂, I, etc. [4,5]. Since *C. tubulosa* has been used in the folk medicine for several years, it is beneficial to find out the biological activities of the individual compounds present in the *C. tubulosa* extracts. In order to establish the detailed medicinal potential of individual components, it is essential to have higher quantities of the compounds, which are difficult to isolate from the plant source. Therefore, development of concise chemical synthetic strategies would be the best option to gain access to these compounds on a large scale. A few reports are available in the literature for the synthesis of phenylethyl oligosaccharides [8,9]. In this context, we developed a synthetic strategy for the synthesis of the common trisaccharide core of kankanose, kankanoside F, H₁, H₂ and I isolated from *C. tubulosa* thereby...
exploiting newly developed regio- and stereoselective glycosylation conditions (Figure 1). This straightforward synthetic strategy employs a minimum number of steps.

Results and Discussion

The target trisaccharide 1 in the form of its 2-phenylethyl glycoside was synthesized from the suitably functionalized monosaccharide derivatives 2 [10], 3 [11], and 4 [12], which were prepared from the commercially available reducing sugars (Figure 1). The key features of this synthetic strategy are (a) the application of two regioselective glycosylations by using glycosyl acceptors with two hydroxy groups; (b) application of molecular iodine to the functional group transformations [13]; and (c) activation of the glycosyl trichloroacetimidate derivative by using nitrosyl tetrafluoroborate (NOBF₄) [14].

The treatment of D-glucose pentaacetate with 2-phenylethanol in the presence of boron trifluoride diethyl etherate furnished 2-phenylethyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (2) in 84% yield [10]. Saponification of compound 2 by using 0.1 M sodium methoxide in methanol followed by benzaldehyde dimethylethel in the presence of molecular iodine [13] furnished compound 5 in 86% yield. Regioselective 3-O-glycosylation of compound 5 with L-rhamnose derived trichloroacetimidate derivative 3 [11] in the presence of NOBF₄ [14] followed by acetylation in the same pot furnished disaccharide derivative 6 in 76% yield. In this case, NOBF₄ acts as a promoter for the activation of the glycosyl trichloroacetimidate derivative as well as the acetylation of the sugar derivative with acetic anhydride. The formation of compound 6 was confirmed by its spectral analysis [signals at δ 5.44 (s, PhCH), 4.79 (br s, H-1_B), 4.37 (d, J = 7.5 Hz, H-1_A) in the 1H NMR and at δ 101.9 (PhCH), 101.3 (C-1_A), 97.4 (C-1_B) in the 13C NMR spectra]. Removal of the benzylidene acetel group under neutral conditions by using triethylsilane and Pd/C [15] resulted in the formation of disaccharide diol 7 in 80% yield. NOBF₄ catalyzed regio- and stereoselective 6-O-glycosylation of compound 7 with D-glucose-derived trichloroacetimidate derivative 4 [12] furnished trisaccharide derivative 8 in 71% yield, which was confirmed by the spectral analysis [signals at δ 4.75 (br s, H-1_B), 4.54 (d, J = 8.0 Hz, H-1_C), 4.22 (d, J = 8.0 Hz, H-1_A) in the 1H NMR and at δ 100.8 (C-1_A), 100.5 (C-1_C), 98.8 (C-1_g) in the 13C NMR spectra]. Saponification of compound 8 by using 0.1 M sodium methoxide in methanol furnished compound 1 in 94% yield. Spectral analysis of compound 1 unambiguously confirmed its formation [signals at δ 5.16 (br s, H-1_B), 4.37 (d, J = 7.5 Hz, H-1_A), 4.32 (d, J = 8.0 Hz, H-1_C) in the 1H NMR and at δ 103.5 (C-1_A), 102.9 (C-1_C), 101.3 (C-1_B) in the 13C NMR spectra] (Scheme 1).

Conclusion

In summary, a straightforward synthetic strategy was developed for the prompt synthesis of a common trisaccharide core of the kankanose, kankanoside F, H₁, H₂ and I isolated from the extract of C. tubulosa. All the steps are high yielding, and glycosylations were highly regio- and stereoselective. Because of the simplicity of the synthetic strategy, it can be applied in a scaled-up preparation.

Experimental

General methods

All reactions were monitored by thin-layer chromatography with silica-gel-coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% Ce(SO₄)₂ in 2 N H₂SO₄) sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. 1H and 13C NMR spectra were recorded on a Bruker Avance 500 MHz by using CDCl₃ as the solvent and TMS as the internal reference unless stated otherwise. Chemical shifts δ are expressed in parts per million (ppm). ESIMS were recorded on a Micromass mass spectrometer. Optical rotations were recorded in a Jasco P-2000 spectrometer. Commercially available grades of organic solvents of adequate purity were used in all reactions.

Figure 1: Structure of the synthesized trisaccharide core found in kankanose, kankanoside F, H₁, H₂ and I isolated from Cistanche tubulosa.
2-Phenylethyl 4,6-O-benzylidene-β-D-glucopyranoside (5): A solution of compound 2 (5.0 g, 11.05 mmol) in 0.1 M CH₃ONa in CH₃CN (10 mL) was stirred at room temperature for 3 h and was neutralized with Amberlite IR-120 (H⁺) resin. The reaction mixture was filtered and concentrated under reduced pressure.

To a solution of the deacetylated product (3.1 g) in CH₃CN (10 mL) was added benzaldehyde dimethyl acetal (2.5 mL, 17.53 mmol) dropwise, and the reaction mixture was stirred for 17.5 h. The reaction mixture was filtered and concentrated under reduced pressure to give the crude product, which was purified over SiO₂ by using hexane/EtOAc (2:1) as an eluant to give pure compound 5 (3.5 g, 86%). Yellow oil; [α]D25 +60 (c 1.2, CHCl₃).

2-Hydroxy-2,3,4-tri-O-acetyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (3): To a stirred solution of compound 1 (3.0 g, 8.06 mmol) and compound 2 (3.6 g, 8.28 mmol) in anhydrous CH₂Cl₂ (15 mL) was cooled to −20 °C under argon. To the cooled reaction mixture was added NOBF₄ (1.0 g, 8.56 mmol), and the reaction mixture was stirred at the same temperature for 20 min. After consumption of the starting material (TLC; hexane/EtOAc 4:1), acetic anhydride (3 mL) was added to the reaction mixture, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic layer was washed with satd. NaHCO₃ and water, dried (Na₂SO₄) and concentrated to a crude product, which was purified over SiO₂ by using hexane/EtOAc (3:1) as an eluant to give the pure product 6 (4.2 g, 76%). Yellow oil; [α]D25 +60 (c 1.2, CHCl₃).

2-Phenylethyl 4,6-O-benzylidene-β-D-glucopyranoside (5): A solution of compound 2 (5.0 g, 11.05 mmol) in 0.1 M CH₃ONa in CH₃CN (10 mL) was stirred at room temperature for 3 h and was neutralized with Amberlite IR-120 (H⁺) resin. The reaction mixture was filtered and concentrated under reduced pressure to give the crude product, which was purified over SiO₂ by using hexane/EtOAc (2:1) as an eluant to give pure compound 5 (3.5 g, 86%). White solid; mp 158–160 °C (EtOH); [α]D25 +60 (c 1.2, CHCl₃).

1H NMR (500 MHz, CDCl₃) δ 7.39–7.11 (m, 10H, Ar-H), 5.44 (s, 1H, PhCH₂), 5.23 (dd, J = 10.0, 3.5 Hz, 1H, H-3β), 4.97 (t, J = 8.0 Hz, 1H, H-2α), 4.89 (br s, 1H, H-2β), 4.85 (t, J = 10.0 Hz, 1H, H-3α), 4.79 (br s, 1H, H-1β). 3.7 (d, J = 7.5 Hz, 1H, H-1α), 4.27 (dd, J = 10.5, 5.0 Hz, 1H, H-6α), 4.04–3.97 (m, 2H, H-5α, H-5β), 3.78 (t, J = 9.5 Hz, 1H, H-1β), 3.69 (t, J = 10.0 Hz, 1H, H-6β), 3.60–3.54 (m, 2H, H-4α, H-4β), 3.39–3.26 (m, 1H, H-5γ), 2.81–2.78 (m, 2H, CH₂), 2.01, 1.91, 1.89, 1.88 (8 s, 12H, 4 COCH₃), 0.59 (d, J = 6.0 Hz, 3H, CCH₃); 13C NMR (125 MHz, CDCl₃) δ 170.0, 169.9, 169.8, 169.4 (4 COCH₃), 138.5–126.3 (Ar-C), 101.9 (PhCH), 101.3 (C-1α), 97.4 (C-1β), 79.0 (C-4α), 76.8 (C-3α), 73.3 (C-2α), 71.3 (C-4β), 70.6 (C-2β), 70.5 (C-4γ), 68.7 (C-6α), 68.4 (C-3γ), 66.6 (C-5β), 66.2 (C-5α), 36.0 (CH₂), 20.9, 20.8, 20.7, 20.6 (4 COCH₃), 16.5 (CH₃); ESIMS: 709.2 [M + Na]+. Anal. calc. for C₃₅H₄₅O₁₄: C, 61.22; H, 6.16; found: C, 61.05; H, 6.35.

2-Phenylethyl 4,6-O-benzylidene-β-D-glucopyranoside (5): To a stirred solution of compound 2 (4.0 g, 5.82 mmol) and 10% Pd/C (0.5 g) in CH₃OH/CH₂Cl₂ (15 mL, 1:1, v/v) was added Et₃SiH (2.8 mL, 17.53 mmol) dropwise, and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was filtered.

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Scheme 1: Reagents: (a) 0.1 M CH₃ONa, CH₃OH, room temperature, 3 h, 98% for compound 5, 94% for compound 1; (b) PhCH(OCH₃)₂, I₂, CH₃CN, room temperature, 1.5 h, 88%; (c) NOBF₄, CH₂Cl₂, −20 °C, 20 min; (d) acetic anhydride, room temperature, 1 h, 76% in two steps; (e) Et₃SiH, 10% Pd/C, CH₃OH/CH₂Cl₂ (1:1, v/v), room temperature, 30 min, 80%; (f) NOBF₄, CH₂Cl₂, −35 °C, 30 min, 71%.

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![Diagram of chemical reactions and structures](image-url)
through a Celite® bed, and the filtering bed was washed with CH₂Cl₂ (50 mL). The combined filtrate was concentrated under reduced pressure to give the crude product, which was purified over SiO₂ by using hexane/EtOAc (1:1) as an eluant to give pure compound 7 (2.8 g, 80%). Yellow oil; [α]D<sub>25</sub> = –30 (c 1.2, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.28–7.18 (m, 5H, Ar-H); 5.23 (dd, J = 10.0, 3.5 Hz, 1H, H-3'); 5.05–5.09 (m, 1H, H-2'B); 4.94 (t, J = 8.0 Hz, 1H, H-2'A); 4.87 (d, J = 1.8 Hz, 1H, H-1'β); 4.41 (d, J = 8.0 Hz, 1H, H-1'A), 4.16–4.06 (m, 2H, CH₂); 3.92–3.86 (m, 1H, H-5'A); 3.58–3.54 (m, 1H, H-5'A), 3.29–3.25 (m, 1H, H-5'), 2.91–2.83 (m, 2H, CH₂); 2.13, 2.04, 2.01, 1.98 (4 s, 12H, 4 COCH₃), 1.21 (d, J = 6.0 Hz, 3H, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.0, 169.4, 169.9, 167.5 (4 COCH₃), 138.5–126.3 (Ar-C), 100.7 (C-1), 98.8 (C-1β), 84.8 (C-4'A), 75.2 (C-3''), 71.4 (C-2''), 70.7 (C-4''β), 70.4 (C-2''β), 69.9 (2C, C-5''β, CH₂), 68.6 (C-2''α), 67.7 (C-5''α), 62.2 (C-6''α), 36.0 (CH₂), 20.9, 20.8, 20.7, 20.6 (4 COCH₃), 17.4 (CH₃); ESIMS: 621.2 [M + Na⁺]; Anal. calc'd for C₂₂H₃₅O₁₄C: C, 56.18; H, 6.40; found: C, 56.05; H, 6.55.

2-Phenylethyl (2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-(1→6)-2-O-acetyl-β-D-glucopyranoside (8): A solution of compound 8 (2.0 g, 2.15 mmol) in 0.1 M CH₃ONa in CH₃OH (50 mL) was stirred at room temperature for 3 h and neutralized with Amberlite IR-120 (H⁺) resin. The reaction mixture was filtered and concentrated to give the crude product, which was purified over Sephadex® LH-20 gel by using CH₃OH/H₂O (10:1) as an eluant to give pure compound 8 (1.2 g, 94%). White powder; [α]D<sub>25</sub> = –11 (c 1.2, CH₂Cl₂); ¹H NMR (500 MHz, CD₂OD) δ 7.26–7.16 (m, 5H, Ar-H), 5.16 (br s, 1H, H-1'β), 4.37 (d, J = 7.5 Hz, 1H, H-1'A), 4.32 (d, J = 8.0 Hz, 1H, H-1'C), 4.15–4.13 (m, 1H, H-6''α), 4.10–4.04 (m, 1H, CH₂); 3.93–3.92 (m, 1H, H-2'B''), 3.88–3.82 (m, 1H, H-6''β), 3.81–3.73 (m, 2H, H-6''β, CH₂), 3.70 (dd, J = 10.0, 3.5 Hz, 1H, H-3''), 3.68–3.64 (m, 1H, H-6''α), 3.50 (t, J = 10.0 Hz, 1H, H-4''α), 3.48–3.44 (m, 2H, H-4''β, H-5''α), 3.39 (t, J = 10.0 Hz, 1H, H-4''), 3.30–3.35 (m, 4H, H-2''A', H-3''A, H-3''β, H-5''β), 3.21 (t, J = 9.0 Hz, 1H, H-2''), 2.94–2.90 (m, 2H, CH₂), 1.25 (d, J = 6.0 Hz, 3H, CH₃); ¹³C NMR (125 MHz, CD₂OD) δ 138.6–125.8 (Ar-C), 103.5 (C-1''β), 102.9 (C-1''A), 101.3 (C-1''β), 82.6 (C-4''A), 76.6 (2C, C-3''β, C-3''A), 75.6 (C-5''A), 74.3 (C-5''β), 73.7 (C-2''A), 72.6 (C-4''C), 71.0 (C-4''A), 70.9 (C-2''A), 70.6 (CH₂), 70.2 (C-2''β), 68.5 (C-5''β), 68.5 (C-6''A), 61.3 (C-6''A), 35.8 (CH₂), 16.5 (CH₃); ESIMS: 615.2 [M + Na⁺]; Anal. calc'd for C₂₂H₃₄O₁₅S: C, 52.70; H, 6.80; found: C, 52.56; H, 7.0.

Supporting Information

Supporting Information File 1

¹H NMR and ¹³C NMR spectra of compounds 1, 2, 5, 6, 7 and 8.

[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-9-80-S1.pdf]

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References


References


And references therein.


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