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Synthesis of Methyl α-D-Glucopyranosyl-(1-2)-α-D-galactopyranosyl-(1-3)-α-D-glucopyranoside and an Acyclic Analogue thereof for Probing the Carbohydrate-binding Specificity of Bacteriophage φX 174

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Key words: oligosaccharide, synthesis, Salmonella, core lipopolysaccharide

The title trisaccharide was synthesized using methyl 1-thioglycoside building blocks. An acyclic analogue, methyl 3-*O*-(α-D-glucopyranosyl-oxyethyl)-α-D-glucopyranoside, which has an ethylene bridge in place of the galactosyl residue, was also synthesized.

The core region of the lipopolysaccharide from *Salmonella* bacteria has the structure [1] shown in Fig. 1. This oligosaccharide can serve as receptor for bacteriophages at the initial stage of phage infection. Thus, mutant *Salmonella* bacteria lacking certain structural elements in the core region are resistant ([2]: Bruse G, Wohlin R, Lindberg AA; unpublished results), and infection of susceptible strains can be inhibited by core oligosaccharide fragments. To study in more detail the specific binding between bacteriophage φX 174 and the core oligosaccharide, synthetic oligosaccharides were needed. We have now synthesized the trisaccharide, methyl α-D-glucopyranosyl-(1-2)-α-D-galactopyranosyl-(1-3)-α-D-glucopyranoside (**8**), which is expected to be bound by the phage. The "acyclic" analogue, methyl 3-*O*-(α-D-glucopyranosyloxyethyl)-α-D-glucopyranoside (**13**) was also synthesized, the purpose being to compare the binding properties of this more flexible structure with the conformationally rigid **8**.

Results and Discussion

The synthetic strategy adopted was based on methyl 1-thioglycoside intermediates. Thus, the target methyl glycosides **8** and **13** were synthesized from the protected methyl

α-D-Gal*p*-1 α-Hep-1

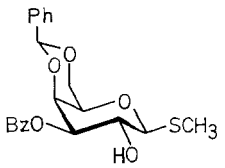
6 7

α-D-GlcNAc*p*-(1-2)-α-D-Glc*p*-(1-2)-α-D-Gal*p*-(1-3)-α-D-Glc*p*-(1-3)-α-Hep-(1-3)-α-Hep

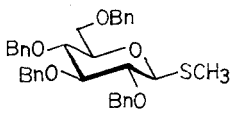
Figure 1. The structure of the Salmonella core oligosaccharide. Hep = L-*glycero*-D-mannoheptopyranoside.

1-thioglycoside trisaccharide precursors **6** and **11**, which were in turn assembled from methyl 1-thioglycoside monosaccharide derivatives. These were prepared in the following way.

Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**1**) was prepared in 60% yield by partial benzoylation of methyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside [3], using benzoyl chloride in pyridine at 0°C. Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**2**) was prepared in 75% yield by treatment of methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside [4] first with methanolic sodium methoxide, then with sodium hydride and benzyl bromide in *N,N*-dimethyl formamide.

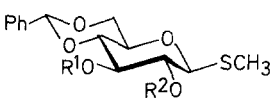


1.



2.

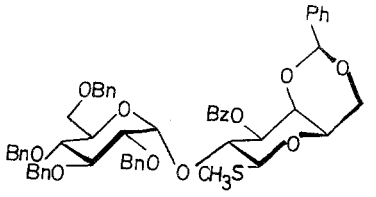
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **3** was prepared in 59% yield by treatment of methyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (Garegg PJ, Fugedi P; unpublished results) with benzyl bromide and tetrabutylammonium hydroxide in a mixture of water and dichloromethane. The corresponding 3-*O*-benzyl ether (**4**) was also formed in 29% yield. The position of benzylation in **3** and **4** was indicated by the downfield NMR shift of H-3 (for **3**) and H-2 (for **4**) on acetylation.



3: $R^1 = H$, $R^2 = Bn$

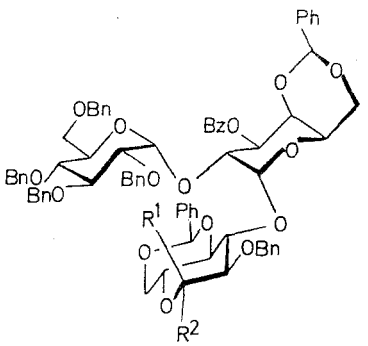
4: $R^1 = Bn$, $R^2 = H$

Silver triflate-promoted glycosidation of **1** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide [5] (prepared from **2** by treatment with bromine) gave the disaccharide **5** in 51% yield. This was treated with bromine in dichloromethane to give a disaccharide



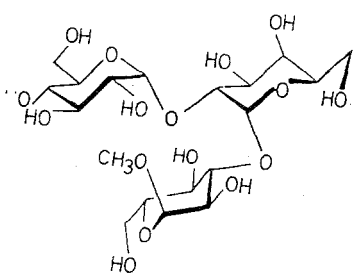
5.

bromide which was used in a silver triflate-promoted glycosidation of **3**. Trisaccharide **6** was obtained (33%). Treatment of **6**, in dichloromethane solution, first with bromine, then with tetraethylammonium bromide and methanol gave the trisaccharide methyl glycoside **7** in 60% yield. Catalytic hydrogenation of **7** followed by treatment with



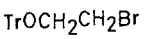
6: $R^1 = H$, $R^2 = SCH_3$
7: $R^2 = H$, $R^1 = OCH_3$

methanolic sodium methoxide gave **8** in 83% yield. The ^{13}C-NMR signals for C-2′ and C-3 (73.7 and 80.3) in **8** were shifted downfield by 4.5 and 6.2 p.p.m., respectively, as compared to the corresponding signals (69.2 and 74.1) of the unsubstituted methyl glycosides [6]. This demonstrates the substitution pattern in **8**.

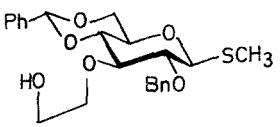


8.

Treatment of **3** with sodium hydride and 1-dimethoxytrityloxy-2-bromoethane (**9**, prepared by dimethoxytritylation of bromoethanol) in *N,N*-dimethylformamide followed by mild acid hydrolysis to remove the dimethoxytrityl group gave **10** in 44% yield.

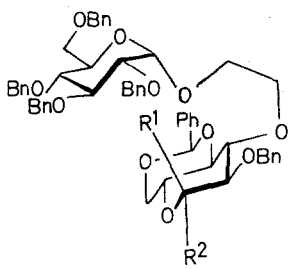


9.



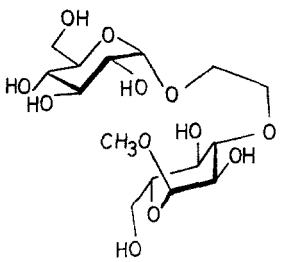
10.

Halide-ion assisted glycosidation of **10** with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide gave **11** (65%). Treatment of **11**, in dichloromethane solution, first with bromine, then with tetraethylammonium bromide and methanol gave the methyl glycoside **12** (56%); catalytic hydrogenation of which gave **13** (57% yield). The ^{13}C-NMR signal for C-3 (83.6) in **13** was shifted downfield by 9.5 p.p.m. as compared to the C-3 signal (74.1) of methyl α-D-glucopyranoside. This demonstrates the substitution pattern in **13**.



11: $R^1 = H$, $R^2 = SCH_3$

12: $R^2 = H$, $R^1 = OCH_3$



13.

Experimental

General Methods

Melting points are corrected. Concentrations were performed at 1-2 kPa at 40°C (bath). Optical rotations were recorded on 0.5-1.0% solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25°C for solutions in C^2HCl_3 unless otherwise stated, using JEOL JNM FX-100 (^{13}C) or GX 400 (^{1}H) instruments. The following reference signals were used: C^2HCl_3 δ 77.17 (^{13}C in C^2HCl_3); $CHCl_3$ δ 7.27 (^{1}H in C^2HCl_3); external Me_4Si δ 0.00 (^{13}C and ^{1}H in ^2H_2O). Only selected NMR data are reported. TLC was performed on Silica Gel F_{254} (Merck Darmstadt, W. Germany) with detection by u.v. light when applicable or by charring with sulphuric acid.

Column chromatography was performed on silica gel 60 (0.04-0.063 mm, Merck) with loadings in the range 1/25-1/100 and elution with toluene/ethyl acetate mixtures unless otherwise stated. Organic solutions were dried over $MgSO_4$. Molecular sieves (4Å, Union Carbide, obtained from Merck) were desiccated under vacuum at 300°C overnight and ground immediately before use.

*Methyl 3-O-Benzoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**1**)*

Benzoyl chloride (2.60 ml) was added dropwise to a stirred and cooled (0°C) solution of methyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside [2] (6.0 g) in pyridine (100 ml). After 2 h, water (0.1 ml) was added, and stirring was continued for 15 min at room temperature. The mixture was partitioned between water and dichloromethane, the organic layer was washed with 1 M sulphuric acid and aqueous sodium hydrogen carbonate, dried, and concentrated. The residue was purified by column chromatography to give **1** (4.68 g, 60%). Crystallisation from dichloromethane-hexane gave material with m.p. 153-155°C, $[\alpha]_D$ + 75°. ^{1}H-NMR data: δ 2.30 (s, SC**H**$_3$), 3.68 (broad s, H-5), 4.06 (dd, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.28 (t, $J_{1,2}$ 9.6, $J_{2,3}$ 9.5 Hz, H-2), 4.39 (dd, H-6b), 4.44 (d, H-1), 4.55 (broad d, $J_{3,4}$ 3.4, $J_{4,5}$ < 1 Hz, H-4), 5.19 (dd, H-3), 5.52 (s, PhC**H**).

Analytical data, calculated for $C_{21}H_{22}O_6S$: C, 62.7; H, 5.5; S, 8.0. Found: C, 62.2; H, 5.5; S, 8.0.

*Methyl 2,3,4,6-Tetra-O-benzyl-1-thio-β-D-glucopyranoside (**2**)*

A mixture of methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside [4] (10.1 g) and methanolic sodium methoxide (0.1 M, 100 ml) was stirred at room temperature until homogeneous, then neutralized with Dowex 50 (H^+) resin and concentrated. The residue was dissolved in *N,N*-dimethylformamide (50 ml) and sodium hydride (50% in oil, 5.3 g) was added, followed by benzyl bromide (12.8 ml). After 2 h, sodium hydride (0.5 g) and methanol (0.5 ml) were added. The mixture was then partitioned between water and toluene/ether, 1/1 by vol, and the organic layer was washed twice with water, dried, and concentrated. Purification of the residue by column chromatography gave **2** (11.4 g, 75%). Crystallistation from hexane gave material with m.p. 65-68°C, $[\alpha]_D$ +8°.

Analytical data, calculated for $C_{35}H_{38}O_5S$: C, 73.7; H, 6.7. Found: C, 73.5; H, 6.5.

*Methyl 2-O-Benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**3**) and Methyl 3-O-Benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**4**)*

A mixture of tetrabutylammonium hydrogen sulphate (0.72 g), methyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (Garegg PJ, Fugedi P; unpublished results) (3.0 g), benzyl bromide (1.72 ml), dichloromethane (180 ml) and aqueous sodium hydroxide (5%, 20 ml) was refluxed for 48 h. Methanol (2 ml) was added, and reflux was continued for 1 h. The organic layer was washed with water, dried, and concentrated. Purification of the residue by column chromatography gave first **3** (2.24 g, 59%). Crystallised from dichloromethane-hexane the material had m.p. 132-134°C, $(\alpha]_D$ -37°.

Analytical data, calculated for $C_{21}H_{24}O_5S$: C, 64.9; H, 6.2; S, 8.2. Found: C, 65.0; H, 6.4; S, 8.3.

In the ^{1}H-NMR spectrum of acetylated **3**, only H-3 (t, 5.31) of the ring protons appeared

in the shift region expected for protons geminal to acyloxy groups. This confirms the position of acetylation, and thus the position of benzylation in **3**.

Further elution of the column gave **4** (1.11 g, 29%). Crystallised from dichloromethane-hexane the material had m.p. 157-158°C, $[\alpha]_D$ -45°.

Analytical data, calculated for $C_{21}H_{24}O_5S$: C, 64.9; H, 6.2; S, 8.2. Found: C, 64.8; H, 6.3; S, 8.2.

In the ^{1}H-NMR spectrum of acetylated **4**, only H-2 (t, 5.08) of the ring protons appeared in the shift region expected for protons geminal to acyloxy groups. This demonstrates the position of benzylation in **4**.

*Methyl 3-O-Benzoyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1-thio-β-D-galactopyranoside (**5**)*

Bromine (0.24 ml) in dichloromethane (5 ml) was added to a cooled (0°C) and stirred mixture of **2** (1.80 g), dichloromethane (25 ml), and molecular sieves. After 1 h, the mixture was filtered, concentrated, and co-concentrated three times with toluene. The residue was mixed with dichloromethane (25 ml), **1** (1.00 g), 2,4,6-trimethylpyridine (0.50 ml) and molecular sieves. The mixture was cooled (-25°C) and stirred while a solution of silver triflate (1.20 g) in toluene (15 ml) was added. After 15 min dichloromethane (50 ml) was added and the mixture was filtered. The filtrate was washed with aqueous sodium thiosulphate, water, 1 M sulphuric acid and aqueous sodium hydrogen carbonate, dried, and concentrated. The residue was purified by column chromatography to give **5** (1.11 g, 51%), $[\alpha]_D$ +126°. NMR data: ^{13}C, δ 11.3 (S**C**H$_3$), 96.6 (C-1'), 101.0 (Ph**C**H); ^{1}H, δ 3.54 (dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.4 Hz, H-2'), 4.24 (d, $J_{1,2}$ 9.5 Hz, H-1), 4.46 (t, $J_{2,3}$ 9.5 Hz, H-2), 5.36 (dd, $J_{2,3}$ 9.5, $J_{3,4}$ 3.7 Hz, H-3), 5.50 (s, PhC**H**), 5.81 (d, $J_{1,2}$ 3.7 Hz, H-1').

*Methyl O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-(1-2)-O-(2-O-benzoyl-4,6-O-benzylidene-α-D-galactopyranosyl)-(1-3)-O-(2-O-benzyl-4,6-O-benzylidene-1-thio)-β-D-glucopyranoside (**6**)*

A solution of **5** (1.05 g) in dichloromethane (10 ml) was treated with bromine (0.30 ml) in dichloromethane (7 ml) as described in the preparation of **5**. The residue after co-concentration with toluene was mixed with dichloromethane (15 ml), **3** (0.30 g), 2,4,6-trimethylpyridine (0.15 ml), and molecular sieves. A solution of silver triflate (290 mg) in toluene (6 ml) was added while stirring and cooling (-30°C). After 15 min, the mixture was processed as described in the preparation of **5**. The yield, after chromatography, of syrupy **6** was 0.48 g (33%), $[\alpha]_D$ +109°. NMR data: ^{13}C, δ 12.7 (S**C**$_3$), 94.3, 95.8 (C-1', C-1''), 100.4, 101.2 (2 Ph**C**H); ^{1}H, δ 4.44 (dd, $J_{1',2'}$ 3.2, $J_{2',3'}$ 10.7 Hz, H-2'), 4.91 (d, $J_{1'',2''}$ 4.2 Hz, H-1''), 5.08, 5.20 (2 s, PhC**H**), 5.63 (dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 3.4 Hz, H-3'), 5.82 (d, $J_{1',2'}$ 3.2 Hz, H-1').

*Methyl O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-(1-2)-O-(2-O-benzoyl-4,6-O-benzylidene-α-D-galactopyranosyl)-(1-3)-O-(2-O-benzyl-4,6-O-benzylidene)-α-D-glucopyranoside (**7**)*

A 1 M solution of bromine in dichloromethane (0.50 ml) was added to a stirred and cooled (0°C) mixture of **6** (0.38 g), dichloromethane (5 ml), and molecular sieves. After 20 min,

tetraethylammonium bromide (0.25 g) was added, followed by methanol (2.0 ml) and more molecular sieves. After stirring at room temperature for 16 h, pyridine (1 ml) was added, and the mixture was filtered and concentrated. Purification by column chromatography gave **7** (0.22 g, 59%), $[\alpha]_D$ +127°. NMR data: ^{13}C, δ 55.4 (OCH_3), 94.3, 95.8 (C-1', C-1''), 98.5 (C-1), 100.5, 101.2 (2 Ph**C**H); ^1H, δ 3.42 (s, OCH_3), 4.53 (dd, $J_{1',2'}$ 3.4, $J_{2',3'}$ 10.8 Hz, H-2'), 4.69 (d, $J_{1,2}$ 4.0 Hz, H-1), 5.00 (d, $J_{1'',2''}$ 3.6 Hz, H-1''), 5.17, 5.39 (2 s, PhC**H**), 5.75 (dd, $J_{2',3'}$ 10.8, $J_{3',4'}$ 3.6 Hz, H-3'), 5.87 (d, $J_{1',2'}$ 3.4 Hz, H-1').

*Methyl O-α-D-Glucopyranosyl-(1-2)-O-α-D-galactopyranosyl-(1-3)-O-α-D-glucopyranoside (**8**)*

A solution of **7** (0.17 g) in 6 ml ethyl acetate/ethanol, 1/2 by vol, was hydrogenated over Pd/C (10%, 100 mg) at 400 kPa for 16 h, then filtered and concentrated. The residue was dissolved in 0.1 M methanolic sodium methoxide (4 ml). After 30 min, the mixture was neutralized with Dowex 50 (H^+) resin, and concentrated. The residue was partitioned between ether and water. The aqueous phase was lyophilized to give pure **8** (0.072 g, 83%), $[\alpha]_D$ +217° (*c* 0.3, water). NMR data: ^{13}C (^2H_2O, 70°C), δ 55.8 (OCH_3), 61.1, 61.3, 61.5 (C-6, 6',6''), 68.4, 70.1, 70.2, 70.6, 70.6, 71.3, 72.1, 72.1, 72.6, 73.5, (C-2,4,5; C-3',4',5'; C-2'',3'',4'',5''), 73.7 (C-2'), 80.3 (C-3), 96.8 (C-1''), 97.3 (C-1'), 100.2 (C-1); ^1H (^2H_2O, 85°C), δ 4.82 (d, *J* 3.8 Hz, H-1), 5.16 (d, *J* 3.8 Hz, H-1''), 5.53 (d, *J* 3.4 Hz, H-1').

*1-Dimethoxytrityloxy-2-bromoethane (**9**)*

Dimethoxytrityl chloride (0.40 g) was added to a stirred and cooled (0°C) solution of 2-bromoethanol (0.08 ml) in pyridine (5 ml). After 2 h, the mixture was concentrated, and the residue purified by column chromatography (toluene/ethyl acetate, 19/1 by vol, containing 1% pyridine). Amorphous **9** was obtained, (0.19 g, 38%). ^{13}C-NMR data: ^{13}C, δ 31.4 (**C**H_2Br), 55.3 (**C**H_3O), 63.8 (**C**H_2), 86.5 (Ph_3**C**O).

*Methyl 2-O-Benzyl-3-O-hydroxyethyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**10**)*

A solution of **3** (0.20 g) in *N,N*-dimethylformamide (5 ml) was stirred and cooled (0°C) while sodium hydride (40 mg, 50% in oil) was added. When gas evolution had ceased, **9** (0.33 g) was added and stirring was continued at room temperature for 16 h. Methanol (0.1 ml) was added, and the mixture was partitioned between water and toluene/ether, 1/1 by vol. The aqueous phase was washed with toluene/ether, and the combined organic phases were dried and concentrated. The residue was dissolved in 80% aqueous acetic acid and then, after 15 min at room temperature, concentrated. The residue was purified by column chromatography to give **10** (0.10 g, 44%). Crystallisation from dichloromethane-hexane gave material with m.p. 124-127°C, $[\alpha]_D$ -36°. NMR data: ^{13}C, δ 13.3 (S**C**H_3), 62.5 (**C**H_2OH), 68.8, 70.5, 74.7, 76.0, 81.0, 81.3, 83.3, 86.5 (C-1,2,3,4,5,6, Ph**C**H_2O, **C**H_2O), 100.4 (Ph**C**H); ^1H, δ 2.18 (s, SC**H**$_3$), 4.39 (d, $J_{1,2}$ 9.8 Hz, H-1), 5.48 (s, PhC**H**).

*Methyl 2-O-Benzyl-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyloxyethyl)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**11**)*

Treatment of **2** (0.60 g) with bromine as described previously gave a crude bromide, which was mixed with 1,2-dichloroethane (10 ml), **11** (0.28 g), tetraethylammonium

bromide (0.24 g) and molecular sieves. After stirring for 24 h at room temperature, the mixture was filtered, washed with aqueous sodium hydrogen carbonate, and concentrated. Purification of the residue by column chromatography gave **11** (0.38 g, 65%), $[\alpha]_D$ +5°. NMR data: ^{13}C, δ 13.2 (S**C**H$_3$), 97.2 (C-1'), 101.1 (Ph**C**H); ^{1}H, δ 2.20 (s, SC**H**$_3$), 4.32 (d, $J_{1,2}$ 9.6 Hz, H-1), 4.83 (d, $J_{1',2'}$ 3.6 Hz, H-1'), 5.48 (s, PhC**H**).

*Methyl 2-O-Benzyl-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyloxyethyl)-4,6-O-benzylidene-α-D-glucopyranoside (**12**)*

A solution of **11** (0.38 g) in dichloromethane (5 ml) was treated first with a 1 M solution of bromine in dichloromethane (0.60 ml), then with tetraethylammonium bromide (0.20 g) and methanol (2 ml), essentially as described for the preparation of **7**. Purification by column chromatography gave **12** (0.21 g, 56%), $[\alpha]_D$ +16°. ^{13}C-NMR data: δ 55.3 (O**C**H$_3$), 97.1 (C-1'), 99.4 (C-1), 101.2 (Ph**C**H).

*Methyl 3-O-(α-D-Glucopyranosyloxyethyl)-α-D-glucopyranoside (**13**)*

A solution of **12** (0.115 g) in 5 ml ethanol/ethyl acetate, 4/1 by vol, was hydrogenated as described for the preparation of **8**. The mixture was filtered to remove catalyst, and concentrated. The residue was dissolved in water and applied to a column of Bio-Gel P2 (Bio-Rad, Richmond, CA, USA). Elution with water gave **13** (0.030 g, 57%), $[\alpha]_D$ +134°. NMR data (^{2}H$_2$O, 25°C): ^{13}C, δ 56.2 (O**C**H$_3$), 61.7, 61.8 (C-6, C-6'), 70.3, 70.8, 72.1, 72.5, 72.8, 73.1, 74.3, (C-2,4,5; C-2',3',4',5'), 68.6, 72.9 (**C**H$_2$O), 83.6 (C-3), 99.6 (C-1'), 100.5 (C-1); ^{1}H, δ 3.42 (s, OC**H**$_3$), 4.80 (d, $J_{1,2}$ 3.6 Hz, H-1), 4.95 (d, $J_{1',2'}$ 3.9 Hz, H-1').

Acknowledgements

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