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Synthesis of the Tetrasaccharide Repeating Unit of the Antigen from Escherichia coli O126 as Its Methyl Glycoside

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**SYNTHESIS OF THE TETRASACCHARIDE REPEATING UNIT OF
THE ANTIGEN FROM *ESCHERICHIA COLI* O126 AS ITS
METHYL GLYCOSIDE**

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ABSTRACT

The tetrasaccharide repeating unit (17) of the antigen from *E. coli* O126 has been synthesized as its methyl glycoside by sequential addition of monosaccharide derivatives. The formation of the β -mannosidic linkage was achieved by Swern oxidation of the glucose derivative followed by reduction of the product with sodium borohydride.

INTRODUCTION

Escherichia coli is a complex group of gram-negative bacteria and many of its serotypes are important human pathogens causing extraintestinal and intestinal infections.¹⁻³ The serological classification of *E. coli* is mainly based on the nature of O-antigens, i.e., the O-specific polysaccharide part of the lipopolysaccharide (LPS), which is the major outer membrane component of the bacteria.⁴ It was reported that natural

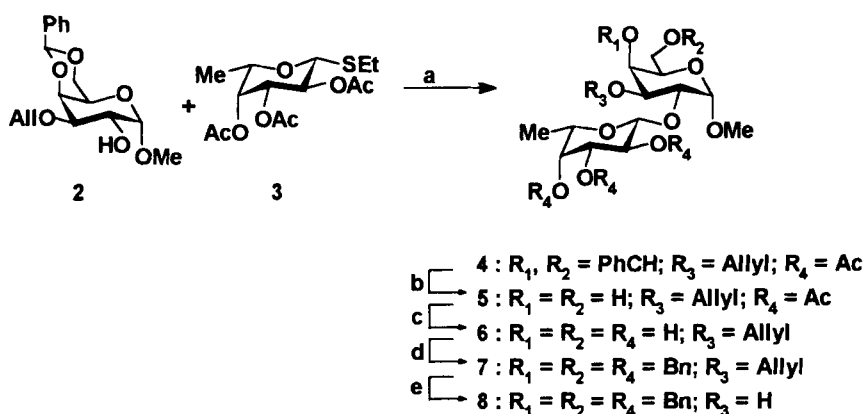
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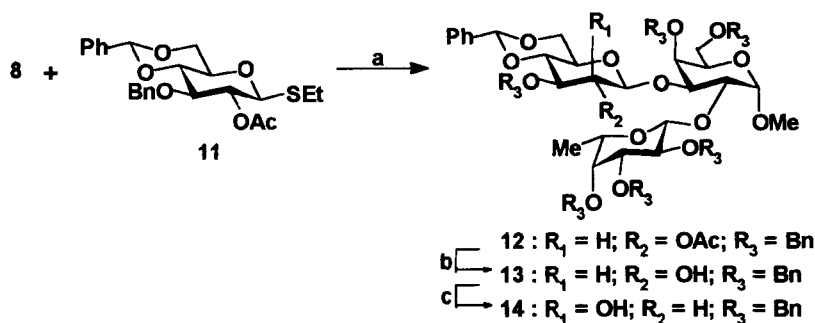
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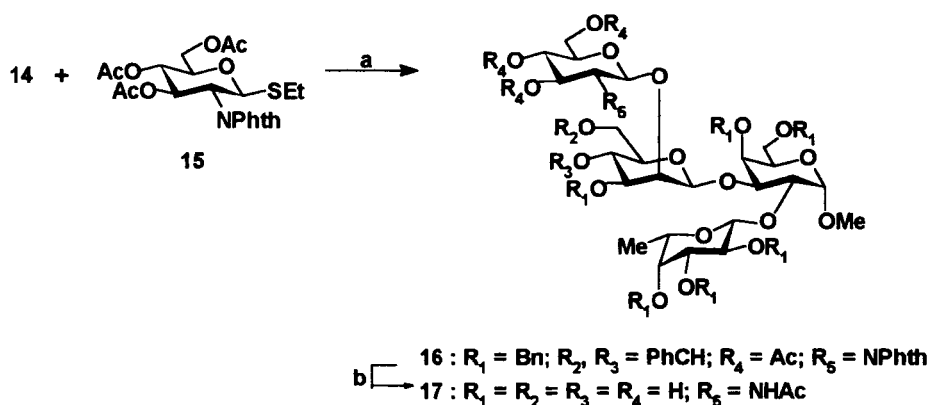
Scheme 1: (a) NIS/TfOH/CH₂Cl₂/MS-4Å/-20 °C/25 min; (b) 85% AcOH/80 °C/2 h; (c) 0.05 M MeONa/MeOH/r t /3 h; (d) BnBr/NaH/DMF/r t/6 h; (e) PdCl₂/MeOH/r t/4 h.

In a separate experiment, ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**9**)¹⁶ was selectively 3-*O*-benzylated¹⁷ using dibutyltin oxide and cesium fluoride giving **10**, which was then acetylated¹⁸ to afford **11**. The disaccharide acceptor **8** was allowed to react with **11** in the presence of NIS/TfOH as promoter¹¹ to give the trisaccharide **12** in 69% yield. Deacetylation¹³ of **12** gave crystalline **13** with a hydroxyl group at the 2'' position. Swern oxidation¹⁹ of **13** using dimethyl sulfoxide-acetic anhydride followed by reduction of the product with sodium borohydride afforded the acceptor **14** in 62% yield (Scheme 2).



Scheme 2 : (a) NIS/TfOH/CH₂Cl₂/MS-4Å/-25 °C/20 min; (b) 0.05 M MeONa/MeOH/r t/8 h; (c) (i) 2:1 Me₂SO-Ac₂O, (ii) NaBH₄/1:1 MeOH-CH₂Cl₂/r t/6 h.

The trisaccharide acceptor **14** was then allowed to react with ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside (**15**)²⁰ in the presence of NIS/TfOH as promoter¹¹ to give the tetrasaccharide derivative **16** in 64% yield. The ¹H NMR spectrum of **16** gave signals at δ 4.78, 4.67, 4.62 and 5.53 ppm for H-1, H-1', H-1'' and H-1''' respectively. Dephthaloylation of **16** with hydrazine hydrate²¹ followed by N-acetylation and hydrogenolysis of the product afforded the target tetrasaccharide repeating unit as its methyl glycoside (**17**) in 67% yield (Scheme 3). The ¹H NMR spectrum of **17** showed anomeric signals for one α -D-galactopyranosyl (δ 4.70), one β -L-fucopyranosyl (δ 4.30), one β -D-mannopyranosyl (δ 4.16) and one 2-acetamido-2-deoxy- β -D-glucopyranosyl (δ 4.83) moiety, together with a peak for a methyl glycoside anomeric proton.



Scheme 3 : (a) NIS/TfOH/ CH_2Cl_2 /MS-4Å/ $-20\text{ }^\circ\text{C}$ /30 min; (b) (i) 10% Pd-C/ H_2 /AcOH/ t /48 h, (ii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ /EtOH/ $90\text{ }^\circ\text{C}$ /2 h, (iii) Pyridine/ Ac_2O / t /4 h, (iv) 0.05 M MeONa/MeOH/4 h.

EXPERIMENTAL

General Procedures. All reactions were monitored by TLC on Silica Gel G (E. Merck, India). Column chromatography was performed using silica gel (SRL, India) and all concentrations were conducted below $50\text{ }^\circ\text{C}$ unless stated otherwise. Optical rotations were measured at $24\text{ }^\circ\text{C}$ with a Perkin-Elmer 241 MC polarimeter. The ¹H and ¹³C NMR spectra were recorded with

Bruker DPX 300 instrument using CDCl_3 as solvent and TMS as the internal standard unless stated otherwise.

Methyl 3-*O*-Allyl-4,6-*O*-benzylidene- α -D-galactopyranoside (2). To a solution of **1** (6.14 g, 26.24 mmol) in CH_3CN (60 mL), were added benzaldehyde dimethyl acetal (6 mL, 39.5 mmol), *p*-TsOH (150 mg) and MS-3Å (7 g) and the mixture was then stirred at room temperature for 16 h. The reaction was quenched with Et_3N (0.5 mL), and the reaction mixture was filtered through a Celite bed and concentrated. The crude product was crystallized from EtOH to give **2** (6.4 g, 76%): mp 158-159 °C; $[\alpha]_{\text{D}} +191.8^\circ$ (*c* 1.09, CHCl_3); ^1H NMR δ 3.43 (s, 3H, OCH_3), 4.82 (d, 1H, $J=3.2$ Hz, H-1), 5.50 (s, 1H, PhCH), 6.0 (m, 1H, $\text{CH}_2\text{CH}=\text{CH}_2$), 7.30-7.56 (m, 5H, aromatic protons).

Anal. Calcd for $\text{C}_{17}\text{H}_{22}\text{O}_6$: C, 63.34; H, 6.88. Found : 63.13; H, 7.08.

Methyl 2,3,4-Tri-*O*-acetyl- β -L-fucopyranosyl-(1 \rightarrow 2)-3-*O*-allyl-4,6-*O*-benzylidene- α -D-galactopyranoside (4). To a solution of ethyl 2,3,4-tri-*O*-acetyl-1-thio- β -L-fucopyranoside **3** (1.95 g, 5.84 mmol), **2** (1.26 g, 3.9 mmol) in CH_2Cl_2 (40 mL), MS-4Å (6 g) was added and the mixture was stirred under Ar at room temperature for 18 h. NIS (1.72 g, 7.65 mmol) was then added, the mixture was allowed to cool to -25°C and TfOH (67 μL , 0.76 mmol) was injected into the cooled reaction mixture. Stirring was continued at -20°C for 20 min, the reaction mixture was diluted with CH_2Cl_2 and filtered through a Celite bed. The filtrate was washed with 10% $\text{Na}_2\text{S}_2\text{O}_3$, aq NaHCO_3 , water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography using 4:1 toluene-Et₂O then gave **4** (2.9 g, 72%); $[\alpha]_{\text{D}} + 50.3^\circ$ (*c* 0.9, CHCl_3); ^1H NMR δ 1.18 (d, 3H, $J=6.1$ Hz, CCH_3), 1.97, 2.06 and 2.14 (3s, 9H, 3COCH_3), 3.40 (s, 3H, OCH_3), 4.65 (d, 1H, $J=7.8$ Hz, H-1'), 4.81 (d, 1H, $J=3.3$ Hz, H-1), 5.54 (s, 1H, PhCH), 5.98 (m, 1H, $\text{CH}_2\text{CH}=\text{CH}_2$), 7.26-7.54 (m, 5H, aromatic protons).

Anal. Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{13}$: C, 58.58; H, 6.40. Found: C, 58.40; H, 6.63.

Methyl 2,3,4-Tri-*O*-benzyl- β -L-fucopyranosyl-(1 \rightarrow 2)-3-*O*-allyl-4,6-di-*O*-benzyl- α -D-galactopyranoside (7). A solution of **4** (2.8 g, 4.71 mmol)

in 85% AcOH (50 mL) was stirred at 90 °C for 3 h. Removal of the solvent gave **5** (2.14 g; 90%) which was treated with 0.05 M MeONa in MeOH (40 mL). After 2 h, the solution was made neutral by the addition of Amberlite IR-120 (H⁺) resin, filtered and the solvent was evaporated to provide **6** (2.1 g, 96%). To a solution of **6** (1.6 g, 4.21 mmol) in DMF (30 mL) were added NaH (50% oil coated, 2.0 g, 41.6 mmol) and BnBr (3.75 mL, 31.5 mmol), and the mixture was stirred at room temperature for 6 h. MeOH (3 mL) was then added to destroy the excess reagents, the reaction mixture was diluted with CH₂Cl₂ (150 mL) and washed with water, dried (Na₂SO₄) and concentrated to give a crude syrup. Column chromatography using 10:1 toluene-Et₂O gave **7** (2.44 g, 70%): [α]_D + 1.05° (c 0.9, CHCl₃); ¹H NMR δ 1.21 (d, 3H, J=6.2 Hz, CCH₃), 3.30 (s, 3H, OCH₃), 4.66 (d, 1H, J=7.2 Hz, H-1'), 4.88 (d, 1H, J=3.3 Hz, H-1), 5.96 (m, 1H, CH₂CH=CH₂), 7.19-7.40 (m, 25H, aromatic protons).

Anal. Calcd for C₅₁H₅₈O₁₀ : C, 73.73; H, 6.98. Found: C, 73.54; H, 7.19.

Methyl 2,3,4-Tri-O-benzyl- β -L-fucopyranosyl-(1 \rightarrow 2)-4,6-di-O-benzyl- α -D-galactopyranoside (8). A mixture of **7** (2.1 g, 2.53 mmol) and PdCl₂ (149 mg, 0.84 mmol) in dry MeOH (30 mL) was stirred at room temperature for 4 h. The reaction mixture was filtered through a celite bed and the filtrate was concentrated. Column chromatography of the crude product using 8:1 toluene-Et₂O gave pure **8** (1.8 g, 90%): [α]_D +13.6° (c 0.8, CHCl₃); ¹H NMR δ 1.23 (d, 3H, J=6.3 Hz, CCH₃), 3.31 (s, 3H, OCH₃), 4.66 (d, 1H, J=7.5 Hz, H-1'), 4.83 (d, 1H, J=3.6 Hz, H-1), 7.24-7.37 (m, 25H, aromatic protons).

Anal. Calcd for C₄₈H₅₄O₁₀ : C, 72.91; H, 6.83. Found: C, 72.76; H, 7.05.

Ethyl 3-O-Benzyl-4,6-O-benzylidene-1-thio- β -D-glucopyranoside (10). A mixture of ethyl 4,6-O-benzylidene-1-thio- β -D-glucopyranoside **9** (2.73 g, 8.75 mmol), dibutyltin oxide (2.6 g, 10.44 mmol) in MeOH (50 mL) was refluxed. After 1 h the reaction mixture became clear, and the solution was concentrated. The residue was dried under vacuum and dissolved in DMF (30 mL). To this solution, predried CsF (1.6 g, 10.53 mmol) and BnBr (1.6 mL, 13.47 mmol) were added and the mixture was stirred at room

temperature for 20 h. The reaction mixture was concentrated, the residue was partitioned between CH_2Cl_2 and water, and the organic layer was dried (Na_2SO_4) and concentrated to a solid mass. Column chromatography using 6:1 toluene- Et_2O gave pure **10** (3.0 g, 85%), which was crystallized from EtOH : mp 145-146 °C; $[\alpha]_{\text{D}} -54.5^\circ$ (c 1.0, CHCl_3); ^1H NMR δ 1.30 (t, 3H, SCH_2CH_3), 2.74 (q, 2H, SCH_2CH_3), 4.45 (d, 1H, $J=9$ Hz, H-1), 4.79, 4.98 (2d, 2H, PhCH_2), 5.56 (s, 1H, PhCH), 7.23-7.44 (m, 10H, aromatic protons).

Anal. Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_5\text{S}$: C, 65.6; H, 6.5. Found: C, 65.8; H, 6.6.

Ethyl 2-O-Acetyl-3-O-benzyl-4,6-O-benzylidene-1-thio- β -D-glucopyranoside (11). Compound **10** (3.0 g, 7.46 mmol) was acetylated conventionally using pyridine and acetic anhydride to give **11** (3.3 g, quantitative): $[\alpha]_{\text{D}} -29.8^\circ$ (c 1.3, CHCl_3); ^1H NMR δ 1.30 (t, 3H, SCH_2CH_3), 2.74 (q, 2H, SCH_2CH_3), 4.45 (d, 1H, $J=9.5$ Hz, H-1), 4.80, 4.98 (2d, 2H, PhCH_2), 5.05 (t, 1H, H-2), 5.56 (s, 1H, PhCH), 7.23-7.44 (m, 10H, aromatic protons).

Anal. Calcd for $\text{C}_{24}\text{H}_{28}\text{O}_6\text{S}$: C, 64.85; H, 6.35. Found: C, 64.74; H, 6.42.

Methyl 2-O-Acetyl-3-O-benzyl-4,6-O-benzylidene- β -D-glucopyranosyl-(1 \rightarrow 3)-[2,3,4-tri-O-benzyl- β -L-fucopyranosyl-(1 \rightarrow 2)]-4,6-di-O-benzyl- α -D-galactopyranoside (12). To a solution of **8** (1.53 g, 1.93 mmol) and **11** (1.28 g, 2.88 mmol) in CH_2Cl_2 (30 mL) was added MS-4Å (5 g) and the reaction was allowed to proceed in presence of NIS (977 mg, 4.34 mmol) and TfOH (33.3 μL , 0.43 mmol) as described for the preparation of **4**. Column chromatography of the crude syrupy product using 10:1 toluene- Et_2O then gave pure **12** (1.56 g, 69%): $[\alpha]_{\text{D}} +2.2^\circ$ (c 1.1, CHCl_3); ^1H NMR δ 1.22 (d, 3H, $J=6.1$ Hz, CCH_3), 1.94 (s, 3H, COCH_3), 3.37 (s, 3H, OCH_3), 4.59 (d, 1H, $J=9.0$ Hz, H-1'), 4.77 (d, 1H, $J=7.6$ Hz, H-1'), 4.93 (d, 1H, $J=2.4$ Hz, H-1), 5.05 (t, 1H, $J=8.7$, H-2'), 5.53 (s, 1H, PhCH), 7.21-7.52 (m, 35H, aromatic protons).

Anal. Calcd for $\text{C}_{70}\text{H}_{76}\text{O}_{16}$: C, 71.67; H, 6.48. Found: C, 71.48; H, 6.65.

Methyl 3-O-Benzyl-4,6-O-benzylidene- β -D-glucopyranosyl-(1 \rightarrow 3)-[2,3,4-tri-O-benzyl- β -L-fucopyranosyl-(1 \rightarrow 2)]-4,6-di-O-benzyl- α -D-galactopyranoside (13). A solution of **12** (1.56 g, 1.33 mmol) was treated

with 0.05 M MeONa in MeOH (50 mL) as described for the preparation of **6** to give a solid mass which crystallized from EtOH yielding **13** (1.39 g, 92%): mp 165-166 °C; $[\alpha]_D +3.1^\circ$ (*c* 1.2, CHCl₃); ¹H NMR δ 1.29 (d, 3H, *J*=6.3 Hz, CCH₃), 3.29 (s, 3H, OCH₃), 4.53 (d, 1H, *J*=8.7 Hz, H-1''), 4.61 (d, 1H, *J*=8.4 Hz, H-1'), 4.84 (d, 1H, *J*=3.3 Hz, H-1), 5.57 (s, 1H, PhCH), 7.20-7.50 (m, 35H, aromatic protons).

Anal. Calcd for C₆₈H₇₄O₁₅: C, 72.21; H, 6.55. Found: C, 72.04; H, 6.73.

Methyl 3-O-Benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→3)-[2,3,4-tri-O-benzyl-β-L-fucopyranosyl-(1→2)]-4,6-di-O-benzyl-α-D-galactopyranoside (14). To a solution of **13** (1.39 g, 1.23 mmol) in Me₂SO (15 mL) was added 1:2 Ac₂O-Me₂SO (30 mL) and the mixture was stirred at room temperature for 16 h. Solvents were removed yielding the 2-keto compound (1.38 g) as a yellow syrup. To a solution of this product in CH₂Cl₂-MeOH (80 mL, 1:1; v/v) was added NaBH₄ (8 g) and the mixture was stirred at 5-10 °C for 5 h. The reaction mixture was concentrated in vacuo and diluted with CH₂Cl₂ (150 mL). The organic layer was washed successively with 5% citric acid solution, aq NaHCO₃, water, dried (Na₂SO₄) and concentrated. Column chromatography using 4:1 toluene-Et₂O gave **14** (0.86 g, 62%) together with its glucoisomer (20%): Compound **14** has $[\alpha]_D +0.9^\circ$ (*c* 1.02, CHCl₃); ¹H NMR δ 1.26 (d, 3H, *J*=6.6 Hz, CCH₃), 3.25 (s, 3H, OCH₃), 4.65 (d, 1H, *J*=9.6 Hz, H-1'), 4.83 (d, 1H, *J*=2.7 Hz, H-1), 5.30 (bs, 1H, H-1''), 5.59 (s, 1H, PhCH), 7.19-7.50 (m, 35H, aromatic protons).

Anal. Calcd for C₆₈H₇₄O₁₅: C, 72.21; H, 6.55. Found: C, 72.08; H, 6.78.

Methyl 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl-(1→3)-[2,3,4-tri-O-benzyl-β-L-fucopyranosyl-(1→2)]-4,6-di-O-benzyl-α-D-galactopyranoside (16). To a solution of **14** (0.86 g, 0.76 mmol) and ethyl 2,3,4-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **15** (0.5 g, 1.14 mmol) in CH₂Cl₂ (30 mL) was added MS-4Å (5 g) and the reaction was allowed to proceed in presence of NIS (385.2 g, 1.71 mmol) and TfOH (15.1

μL , 0.17 mmol) as described for the preparation of **4**. Column chromatography using 5:1 toluene-Et₂O gave **16** (0.75 g, 64%): $[\alpha]_{\text{D}} - 9^\circ$ (c 0.8, CHCl₃); ¹H NMR δ 1.13 (d, 3H, J=6.2 Hz, CCH₃), 1.89, 1.94 and 1.97 (3s, 9H, 3 COCH₃), 3.21 (s, 3H, OCH₃), 4.62 (bs, 1H, H-1''), 4.67 (d, 1H, J=9.3 Hz, H-1'), 4.78 (d, 1H, J=3.6 Hz, H-1), 5.27 (d, 1H, J=12.3 Hz, H-1'''), 5.42 (s, 1H, PhCH), 7.12-7.87 (m, 39H, aromatic protons); ¹³C NMR δ 17.40 (CCH₃), 21.14, 21.16, 21.22 (3COCH₃), 55.56 (OCH₃), 68.44, 68.97, 69.32 (3 C-6), 70.95, 71.12, 71.24, 72.76, 73.25, 73.67, 74.10, 74.63, 74.92, 75.19, 75.86, 77.84, 78.03, 78.16, 78.68, 79.49, 82.96, 97.38 (C-1), 101.07 (C-1''), 102.09 (C-1'), 103.45 (C-1'''), 103.57 (PhCH), 123.78-140.46 (aromatic carbons), 168.27, 169.91, 170.51, 171.27, 171.32 (5 carbonyl carbons).

Anal. Calcd for C₈₈H₉₃O₂₄N: C, 68.26; H, 6.01. Found: C, 68.09; H, 6.24.

Methyl 2-Acetamido-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-mannopyranosyl-(1 \rightarrow 3)-[β -L-fucopyranosyl-(1 \rightarrow 2)]- α -D-galactopyranoside (17**).** To a solution of **16** (700 mg, 0.45 mmol) in aqueous 95% ethanol (25 mL) was added hydrazine hydrate (2 mL). The mixture was heated at 85 °C for 2 h and then concentrated. The residue was treated with pyridine (10 mL) and Ac₂O (7 mL) at room temperature for 3 h and concentrated. The residue was purified by column chromatography using 5:1 toluene-Et₂O. The product was dissolved in AcOH (5 mL) and stirred under H₂ for 2 days in the presence of 10% Pd-C (500 mg). The reaction mixture was then filtered through a Celite bed, concentrated, dissolved in water (2 mL), passed through a 0.45 μm Millipore membrane and dried to afford **17** (211 mg, 67%): $[\alpha]_{\text{D}} + 26.9^\circ$ (c 0.9, H₂O); ¹H NMR (D₂O) δ 1.11 (d, J=6.3 Hz, 3H, CCH₃), 1.91 (s, 3H, NCOCH₃), 3.27 (s, 3H, OCH₃), 4.16 (bs, 1H, H-1''), 4.30 (d, 1H, J=7.8 Hz, H-1'), 4.70 (bs, 1H, H-1), 4.83 (d, 1H, J= 8.4 Hz, H-1'''); ¹³C NMR (D₂O) δ 16.46 (CCH₃), 23.16 (NCOCH₃), 56.27 (OCH₃), 61.64, 61.70, 62.13 (3 C-6), 67.74, 67.95, 69.46, 70.58, 70.95, 71.71, 72.61, 74.21, 76.32, 76.85, 76.96, 79.44, 99.92 (C-1), 101.17 (C-1''), 102.19 (C-1'), 103.25 (C-1'''), 175.44 (NCOCH₃).

Anal. Calcd for C₂₇H₄₇O₂₀N: C, 45.96; H, 6.66. Found: C, 45.80; H, 6.89.

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