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Note

A convenient synthesis of the branching-point trisaccharide of starch and glycogen

SABINE KÖPPER* AND URI ZEHAVI
The Hebrew University of Jerusalem, Department of Biochemistry and Human Nutrition, Faculty of Agriculture, P.O. Box 12, Rehovot 76100 (Israel)

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The trisaccharide *O*-α-D-glucopyranosyl-(1→4)-*O*-[α-D-glucopyranosyl-(1→6)]-D-glucopyranose represents the branching point of amylopectin and glycogen. Therefore, it is of special interest for studies of enzymes participating in starch and glycogen metabolism and plays an important role in the three-dimensional structure of these natural polymers.

We were interested in obtaining this trisaccharide in the course of our studies on glycogen synthase[1]. Unfortunately, the trisaccharide is not readily available from its natural sources[2] and although several syntheses have been published[3–5], they did not appear suitable for our purposes. Either they have as an object the methyl glycoside of the natural product[3,5] or represent rather lengthy pathways. Consequently, we conceived a shorter and more convenient route leading to a product readily attachable to carrier polymers.

Boron trifluoride-mediated glycosylation of 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (**1**) with 4-methoxycarbonyl 2-nitrobenzyl alcohol[6] (**2**) gave the β-D-glycoside[6] **3** in 75% yield. The alcohol **2** was chosen as an aglycon for its bifunctionality and its photosensitivity, which enables linking to and release from polymers as previously described[6–8]. Glycoside **3** was subsequently deesterified to give **4**, and then selectively protected by the bifunctional 1,1,3,3-tetra-(2-propyl)disiloxanyl group[9] and reacetylated to yield **5**. The last three steps could be carried out as a "one-pot procedure" to give **5** in 57% yield. The reacetylation was improved by the presence of 4-dimethylaminopyridine; otherwise a considerable proportion of a product having a free OH-3 was detected. The formation of such a compound is usual for glycosides protected with the 1,1,3,3-tetraisopropyldisiloxanyl group[9]. Finally, the disiloxan group was split off by treatment with

*Author for correspondence. Present address: Universität Oldenburg, Fachbereich Chemie, Postfach 2503, D-2900 Oldenburg (F.R.G.).

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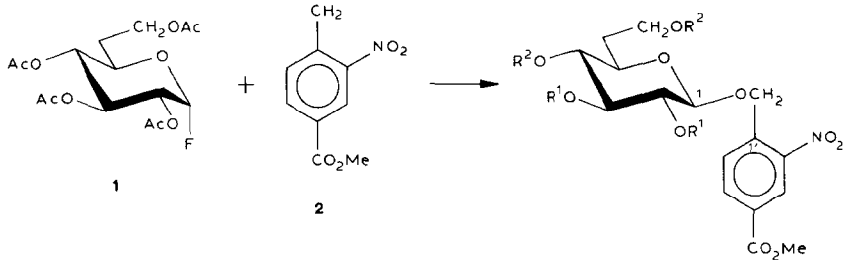
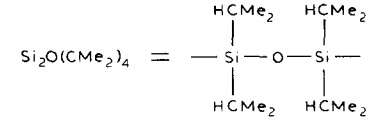
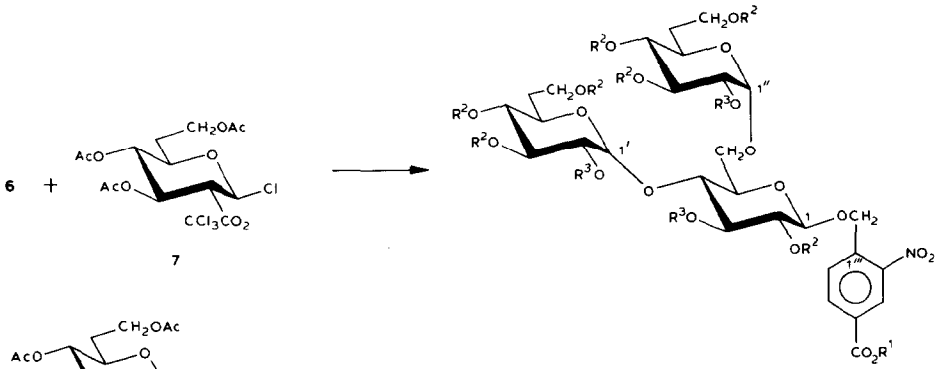


1 2

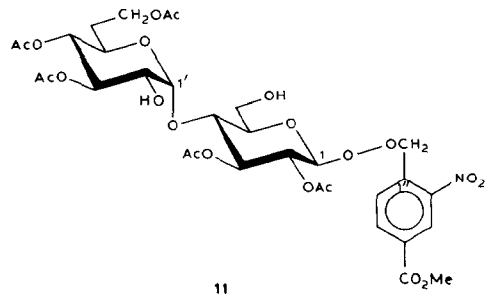
3 $R^1 = R^2 = Ac$
4 $R^1 = R^2 = H$
5 $R^1 = Ac, R^2 = Si_2O(CMe_2)_4$
6 $R^1 = Ac, R^2 = H$





7

8 $R^1 = Me, R^2 = Ac, R^3 = CCH_3CO$
9 $R^1 = Me, R^2 = Ac, R^3 = H$
10 $R^1 = R^2 = R^3 = H$



11

fluoride ions, and condensation of the resulting glycoside (**6**) with the known[10] β-chloride **7** gave trisaccharide **8**.

This glycosylation step, however, proved to be crucial; although t.l.c. indicated almost quantitative formation of **8** in the first experiments, after workup and silica gel separation, only the disaccharide **11** and several monosaccharide derivatives could be isolated. Compound **11** was fully characterized by ^{1}H-n.m.r., but the unequivocal assignment of the linkage position was, however, not possible by chemical-shift differences. Therefore, an n.O.e. experiment was performed which showed the presence of a (1→4)-linkage. T.l.c. suggested that **11** is a

degradation product of the trisaccharide **8**. In the presence of the trichloroacetyl groups, splitting of the glycosidic linkages of **8** occurred nearly quantitatively during workup, the (1→6)-linkage being split off first, since the only isolable product was the (1→4)-linked disaccharide **11**.

In view of these findings, the trichloroacetyl groups were removed prior to any other treatment. Thus, after glycosylation in a highly concentrated mixture, the solution was filtered through silica gel previously treated with triethylamine. By this procedure, trisaccharide **9** was obtained in 57% yield and no other glycosylation products were detected. Compound **9** was characterized by ^{1}H-n.m.r. which showed the expected α-D-(1→4)- and α-D-(1→6)-linkages. Deacetylation and subsequent demethylation gave **10** in good yields; its ^{1}H-n.m.r. spectrum was in good agreement with data for the corresponding methyl glycoside[5].

EXPERIMENTAL

General methods. — Melting points (not corrected) were determined with a Büchi 510 apparatus and optical rotations measured with a Bendix polarimeter. ^{1}H-N.m.r. spectra were recorded with Bruker instruments WM 200, WH 300, and AM 500 at 200, 300, and 500 MHz, respectively. Chemical shifts are given downfield from the signal of tetramethylsilane for solutions in organic solvents. Solutions in D_2O were calibrated with ($^2H_6$)acetone. The n.O.e. experiment was performed in a difference mode with an instrument operating at 300 MHz for a solution in (^{2}H)chloroform at 300 K; irradiation time was 0.8 s and decoupler power 46 L. All reactions were carried out under protection from light and monitored by t.l.c. on Silica gel $60F_{254}$-coated plates (Merck). Components were detected by viewing under u.v. light, and spraying with 10% H_2SO_4 in ethanol and heating. Column chromatography was performed on Silica gel 60 (Merck), and gel chromatography on Bio-Beads SX-2 (Bio-Rad) using toluene as eluent at a velocity of 0.1 mL/min. All solvents were dried according to standard methods.

4-Methoxycarbonyl-2-nitrobenzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside[6] (**3**). — A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride (**1**; 3.87 g, 11 mmol) and 4-methoxycarbonyl-2-nitrobenzyl alcohol (**2**; 2.10 g, 9.9 mmol) in dry dichloromethane (25 mL) was stirred for 1 h with a molecular sieve. After treatment with boron trifluoride ethyl etherate (30%, 2.3 mL) for 2 h, the mixture was diluted with dichloromethane (50 mL) and poured into a cold (0°) solution of $NaHCO_3$. The mixture was extracted with dichloromethane, dried, and concentrated. Crystallization from ethyl acetate–petroleum ether gave **3** (2.12 g). The residue from the residual mother-liquor was chromatographed on silica gel (1:1 ethyl acetate–petrolether) to yield additional **3** (1.94 g; total yield 4.06 g, 75%), m.p. 113°, $[\alpha]_D^{23}$ −25.0° (*c* 2.0, chloroform); lit.[6] m.p. 112–113°, $[\alpha]_D^{25}$ −24.6°.

4-Methoxycarbonyl-2-nitrobenzyl β-D-glucopyranoside (**4**). — Compound **3** (1.49 g, 2.7 mmol) in methanol (90 mL) and dichloromethane (10 mL) was stirred with sodium methoxide (0.1M in methanol, 12 mL) for 15 min, upon which part of

4 crystallized. The mixture was filtered, the residual solution neutralized with Amberlite IR-120 (H^+) ion-exchange resin, and filtered. Evaporation of the solvent gave a crystalline solid, which was combined with the previously obtained crystals to yield **4** (1.01 g, 98%), m.p. 210–212° (dec.), $[\alpha]_D^{19}$ −53° (*c* 0.4, pyridine); ^{1}H-n.m.r. (500 MHz, D_2O): δ 8.53 (d, 1 H, H-3′ aryl), 8.12 (dd, 1 H, $J_{3',5'}$ 1.7 Hz, H-5′ aryl), 7.78 (d, 1 H, $J_{5',6'}$ 8.1 Hz, H-6′ aryl), 5.14 and 5.02 (2 d, each 1 H, *J* 15.2 Hz, $C_6H_3CH_2$), 4.36 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.75 (s, 3 H, OCH_3), 3.68 (dd, 1 H, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.49 (dd, 1 H, H-6b), 3.27 (dd≈t, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 3.23 (ddd≈t, 1 H, $J_{5,6a}$ 2.0, $J_{5,6b}$ 5.5 Hz, H-5), 3.18 (dd≈t, 1 H, $J_{4,5}$ 9.1 Hz, H-4), and 3.16 (dd≈t, 1 H, $J_{2,3}$ 9.0 Hz, H-2).

Anal. Calc. for $C_{15}H_{19}NO_{10}$ (373.3): C, 48.26; H, 5.13; N, 3.75. Found: C, 48.60; H, 5.28; N, 3.95.

*4-Methoxycarbonyl-2-nitrobenzyl 2,3-di-*O*-acetyl-4,6-*O*-[1,1,3,3-tetra(2-propyl)-disiloxan-1,3-yl]-β-*D*-glucopyranoside* (**5**). — A solution of **3** (1.95 g, 3.6 mmol) in methanol (100 mL) and dichloromethane (20 mL) was treated for 45 min with sodium methoxide (0.1M in methanol, 45 mL), followed by neutralization with Amberlite IR-120 (H^+) ion-exchange resin and filtration. The filter paper was washed with pyridine, the filtrate was concentrated, and the residual solvent two times coevaporated with toluene. The resulting syrup was dissolved under N_2 in pyridine (45 mL), the solution cooled to −10° and 1,3-dichloro-1,1,3,3-tetra(2-propyldisiloxane (1.4 mL, 4.4 mmol) added. Stirring was continued for 2–12 h at room temperature when the excess of the reagent was destroyed by addition of 2-propanol (0.05 mL). Stirring was continued for several min, acetic anhydride (30 mL) and 4-dimethylaminopyridine (50 mg) were added, and the solution was kept overnight at room temperature. The molecular sieve was removed by filtration and washed with toluene. The solution was concentrated and the residual solvent three times coevaporated with toluene. The residual syrup was purified by chromatography on silica gel (1:3 ethyl acetate–petrolether) to yield **5** (1.43 g, 57%), $[\alpha]_D^{18}$ −63° (*c* 1.1, chloroform); ^{1}H-n.m.r. (200 MHz, $CDCl_3$): δ 8.72 (d, 1 H, $J_{3',5'}$ 1.7 Hz, H-3′ aryl), 8.28 (dd, 1 H, $J_{5',6'}$ 8.3 Hz, H-5′ aryl), 7.84 (d, 1 H, H-6′ aryl), 5.38 (d, 1 H, *J* 15.8 Hz, $C_6H_3CH_2$), 5.23 (dd≈t, $J_{3,4}$ 9.3 Hz, H-3), 5.03 (d, 1 H, $C_6H_3CH_2$), 5.01 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 4.66 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.14 (dd, 1 H, $J_{6a,6b}$ 12.8 Hz, H-6b), 4.09 (d≈t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 4.03 (dd, 1 H, $J_{5,6b}$ 2.0 Hz, H-6a), 3.98 (s, 3 H, OCH_3), 3.34 (ddd≈dt, 1 H, $J_{5,6a}$ 1.2 Hz, H-5), 2.06 and 2.07 (each s, each 3 H, 2 $OCOCH_3$), and 1.04 (m, 28 H, 4 $HCMe_2$).

Anal. Calc. for $C_{31}H_{49}NO_{13}Si_2$ (699.9): C, 53.20; H, 7.06; N, 2.00. Found: C, 52.82; H, 7.17; N, 1.94.

*4-Methoxycarbonyl-2-nitrobenzyl 2,3-di-*O*-acetyl-β-*D*-glucopyranoside* (**6**). — Compound **5** (1.83 g, 2.6 mmol) and pyridinium hydrochloride (300 mg, 2.6 mmol) were dissolved in freshly distilled oxolane (150 mL) and stirred with molecular sieve for 30 min. Dry M tetrabutylammonium fluoride (3.5 mL, 3.5 mmol) in oxolane was added and stirring continued for 20 min. The mixture was then filtered, the filtrate concentrated, and the residue dissolved in ethyl acetate. The solution

was washed with water, dried (Na_2SO_4), and concentrated to dryness. Purification on a silica gel column (ethyl acetate) gave **6** (713 mg, 60%), $[\alpha]_D^{20}$ −53° (*c* 0.63, chloroform); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 8.72 (d, 1 H, $J_{3,5}$ 1.6 Hz, H-3′ aryl), 8.29 (dd, 1 H, $J_{5',6'}$ 8.1 Hz, H-5′ aryl), 7.83 (d, 1 H, H-6′ aryl), 5.29 and 5.06 (2 d, each 1 H, *J* 15.5 Hz, $C_6H_3CH_2$), 5.06 (mc, 2 H, H-2,3), 4.66 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.98 (s, 3 H, OCH_3), 3.95 (dd, 1 H, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.89 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-6b), 3.84 (dd≈t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.49 (ddd≈dt, 1 H, $J_{5,6a}$ 3.6 Hz, H-5), 2.12 and 2.08 (each s, each 3 H, 2 $OCOCH_3$).

Anal. Calc. for $C_{19}H_{23}NO_{12}$ (475.4): C, 49.89; H, 5.07; N, 3.06. Found: C, 50.32; H, 5.06; N, 2.89.

*4-Methoxycarbonyl-2-nitrobenzyl 2,3-di-*O*-acetyl-4,6-di-*O*-(3,4,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**9**). — The following reaction was carried out under N_2. Compound **6** (450 mg, 0.99 mmol) and silver triflate (1.25 g, 4.7 mmol) in diethyl ether (3 mL) and dichloromethane (3 mL) were stirred for 1 h with pulverized molecular sieve. A solution of **7** (ref. 10; 2.2 g, 4.68 mmol) and 2,4,6-trimethylpyridine (0.6 mL, 4.93 mmol) in dichloromethane (15 mL) was added, and the solution was concentrated to a volume of 7 mL by passing dry N_2 through it. After 20 h at room temperature, the syrupy mixture was diluted with ethyl acetate containing 1% triethylamine and filtered through Celite and silica gel (previously washed with 3% triethylamine–ethyl acetate), followed by extensive washings with 1% triethylamine–ethyl acetate. The resulting solution was washed successively with solutions of $NaHCO_3$, and $Na_2S_2O_3$ water, dried (Na_2SO_4), and concentrated. Chromatographic purification on silica gel (3:1 ethyl acetate–petrol ether), followed by gel permeation chromatography yielded **9** (586 mg, 57%), $[\alpha]_D^{18}$ +29° (*c* 1.7, chloroform); ^{1}H-n.m.r. (500 MHz, $CDCl_3$): δ 8.71 (d, 1 H, $J_{3''',5'''}$ 1.7 Hz, H-3‴ aryl), 8.28 (dd, 1 H, $J_{5''',6'''}$ 8.1 Hz, H-5‴ aryl), 7.81 (d, 1 H, H-6‴ aryl), 5.29 (dd≈t, 1 H, $J_{2',3'}$ 9.3 Hz, H-3′,*), 5.24 (d, 1 H, *J* 15.0 Hz, $C_6H_3CH_2$), 5.23 (dd≈t, 1 H, $J_{2'',3''}$ 9.8 Hz, H-3″,*), 5.14 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 5.12 (dd≈t, 1 H, $J_{3,4}$ 9.0 Hz, H-3), 5.12 (d, 1 H, $C_6H_3CH_2$), 5.08 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 5.01 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1″), 5.00 (dd≈t, 1 H, $J_{3'',4''}$ 10.4 Hz, H-4″), 5.00 (dd≈t, 1 H, $J_{3',4'}$ 9.6 Hz, H-4′), 4.71 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.31 (dd, 1 H, $J_{5',6b'}$ 4.6 Hz, H-6′,**), 4.19 (dd, 1 H, $J_{5'',6b''}$ 4.6 Hz, H-6b″,**), 4.08 (dd, 1 H, $J_{6a',6b'}$ 12.3 Hz, H-6a′,**), 4.07 (dd, 1 H, $J_{6a'',6b''}$ 12.4 Hz, H-6a″,**), 4.04 (ddd, 1 H, $J_{5'',6a''}$ 3.0 Hz, H-5″,***), 3.99 (ddd, 1 H, $J_{5',6a'}$ 2.4 Hz, H-5′,***), 3.99 (dd, 1 H, $J_{6a,6b}$ 12.6 Hz, H-6′), 3.97 (s, 3 H, OCH_3), 3.96 (dd, 1 H, $J_{5,6b}$ 5.8 Hz, H-6b), 3.90 (dd≈t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.34 (ddd, 1 H, $J_{5,6a}$ 2.0 Hz, H-5), 3.26 (mc, 4 H, H-2′,2″), OH-2′ and OH-2″), 2.08, 2.07, 2.06, 2.05, 2.05, 2.04, 2.03, and 2.01 (each s, each 3 H, 8 $OCOCH_3$); assignments marked *, **, *** may be inverted.

Anal. Calc. for $C_{43}H_{55}NO_{28}$ (1033.9): C, 49.95; H, 5.36; N, 1.35. Found: C, 49.48; H, 5.26; N, 1.39.

*4-Carboxy-2-nitrobenzyl 4,6-di-*O*-α-*D*-glucopyranosyl-β-*D*-glucopyranoside* (**10**). — A solution of **9** (501 mg, 0.48 mmol) in methanol (50 mL) was treated with sodium methoxide (0.1M in methanol, 5 mL) for 30 min, and then neutralized with

Amberlite IR-120 (H^+) ion-exchange resin, filtered, and concentrated. The residue was dissolved in water (20 mL) and M NaOH (20 mL) was added. After 30 min, the solution was neutralized as described above, filtered, and concentrated to yield **10** (279 mg, 84%), $[\alpha]_D^{20}$ +32° (*c* 0.3, water); ^{1}H-n.m.r. (500 MHz, D_2O): δ 8.46 (d, 1 H, $J_{3'',5''}$ 1.7 Hz, H-3‴ aryl), 8.10 (dd, 1 H, $J_{5''',6'''}$ 8.0 Hz, H-5‴ aryl), 7.78 (d, 1 H, H-6‴ aryl), 5.22 (d, 1 H, $J_{1'',2''}$ 4.0 Hz, H-1″), 5.11 (s, 2 H, $C_6H_3CH_2$), 5.05 (d, 1 H, $J_{1'',2''}$ 3.8 Hz, H-1″), 4.47 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.50 (dd, 1 H, $J_{2'',3''}$ 10.0 Hz, H-2″), 3.43 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2′), 3.43 (dd, 1 H, *J* 9.7 Hz, H-4″,*), 3.30 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), and 3.27 (dd≈t, 1 H, *J* 9.6 Hz, H-4′,*); assignments marked * may be inverted.

Anal. Calc. for $C_{26}H_{37}NO_{20}$ (683.6): C, 45.68; H, 5.45; N, 2.05. Found: C, 45.58; H, 5.26; N, 1.99.

*4-Methoxycarbonyl-2-nitrobenzyl 2,3-di-*O*-acetyl-4-*O*-(2,4,6-tri-*O*-acetyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**11**). — The following reaction was carried out under N_2. Compound **6** (100 mg, 0.218 mmol) and silver triflate (280 g, 1.04 mmol) in dichloromethane (5 mL) were stirred with pulverized molecular sieve for 45 min. Compound **7** (410 mg, 1.0 mmol) and 2,4,6-trimethylpyridine (0.12 mL) in dichloromethane (4 mL) were added dropwise. After 2 h at room temperature, the solution was diluted with dichloromethane, filtered through Celite and washed successively with dilute $NaHCO_3$, an $Na_2S_2O_3$ solution, and water, dried (Na_2SO_4), and concentrated. The syrup residue was applied to a silica gel column (1:1 ethyl acetate–petrolether). The only oligosaccharide derivative thus isolated was **11** (21 mg, 11%), $[\alpha]_D^{20}$ +4° (*c* 0.5, chloroform); ^{1}H-n.m.r. (300 MHz, C_6D_6): δ 8.58 (d, 1 H, $J_{3'',5''}$ 1.7 Hz, H-3″ aryl), 7.95 (dd, 1 H, $J_{5'',6''}$ 8.3 Hz, H-5″ aryl), 7.60 (d, 1 H, H-6″ aryl), 5.53 (dd≈t, 1 H, $J_{3',4'}$ 9.4 Hz, H-3′), 5.38 (mc, 2 H, H-2,3), 5.24 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 5.16 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, H-4′), 5.15 and 4.83 (2 d, each 1 H, *J* 16.0 Hz, $C_6H_3CH_2$), 4.43 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.08 (mc, 2 H, H-6a′,6b′), 4.04 (mc, 1 H, H-5′), 3.94 (dd≈t, $J_{3,4}$ 9.4, $J_{4,5}$ 9.4 Hz, H-4), 3.84 (dd, 1 H, $J_{5a',6b'}$ 3.8 Hz, H-6′), 3.55 (dd, 1 H, $J_{6a,6b}$ 10.4 Hz, H-6), 3.48 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-2′), 3.41 (s, 3 H, OCH_3), 3.28 (ddd≈dt, $J_{5,6a}$ 3.5, H-5), 1.83, 1.80, 1.77, 1.70, and 1.67 (5 s, each 3 H, 5 $COCH_3$); n.O.e.: irradiation of H-1′ and benzylic-H (70% saturation) gave enhancements at H-2′ (36.1%), H-4 (7.5%), H-1 (5.2%), and benzylic-H′ (51.2%).

Anal. Calc. for $C_{31}H_{39}NO_{20}$ (745.6): C, 49.93; H, 5.27; N, 1.87. Found: C, 50.01; H, 5.29; N, 1.90.

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