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Note

Preparation and biological evaluation of some 1,2-*O*-isopropylidene-D-hexofuranose esters

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Dedicated to the memory of Professor Ivano Morelli

Abstract—The synthesis and biological evaluation of some new glycose esters bearing the 1,2-*O*-isopropylidene-D-hexofuranose functionality and belonging to the 3-*O*-acyl-D-allose and 6-*O*-acyl-D-glucose series are reported. When the results concerning cell growth inhibition are compared, it appears that the 6-*O*-acyl-D-glucose derivatives are more active than the 3-*O*-acyl-D-allose compounds. Within both 6-*O*-acyl-D-glucose and 3-*O*-acyl-D-allose derivatives, butyric esters displayed the highest inhibitory effects. Inhibition of cell growth is not associated with high induction levels of erythroid differentiation, despite the fact that pivaloates induce erythroid differentiation to an extent similar to that exhibited by previously reported molecules [*Bioorg. Med. Chem. Lett.* **1999**, *9*, 3153–3158].

Keywords: K562 Myelogenous leukemia cells; Erythroid differentiation inducers; 6-*O*-Acyl-D-glucofuranoses; 3-*O*-Acyl-D-allofuranoses

Several human tumor cell lines are available and have been used to identify antitumor compounds. Among them, the human chronic myelogenous leukemia K562 cell line has been shown to be a very useful experimental system to correlate antiproliferative activity with the ability to stimulate differentiation along the erythroid pathway.[1] In addition to applications in cancer research and treatment, K562 cells have been used to identify inducers of γ-globin gene expression of possible interest in the therapy of several hematological diseases, including β-thalassemia and sickle cell anemia.[2]

We have recently synthesized and tested on K562 cells several kinds of partially acetonated monosaccharide butyrates and related esters (**1**–**5**), with the aim of establishing the role of the structure of either the ester and the glycose residue on the differentiation activity.[3] Interestingly, while the activities of the different butyrates (**1a**, **2a**, **3a**, **4a**, and **5a**) are strictly analogue and close to that of free butyric acid, isobutyrates and pivaloates display a net difference in their differentiation properties, depending on the type of glycose carrier and also on the location of the ester functionality.[3] On the basis of these results, we made the assumption that these compounds may be considered not only as prodrugs, as previously assumed,[4] but also as active agents. In addition, some of these compounds, in particular the two 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives **1b** and **1c**, potentiate erythroid induction of K562 cells treated with sub-optimal concentrations of cytosine arabinoside, retinoic acid, and mythramycin and were considered of interest for further studies aimed at the inhibition of cell growth in association to activation of differentiated functions.[3]

We are now presenting the results of the extension of our studies to some new glycose esters bearing the

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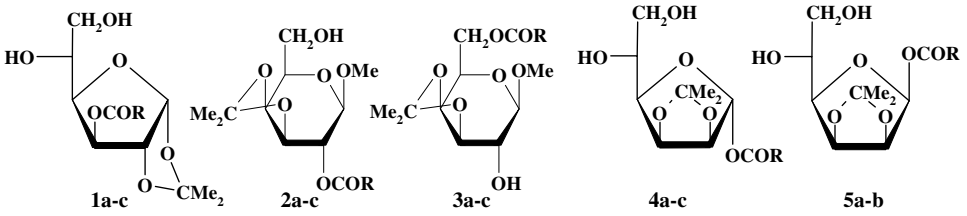
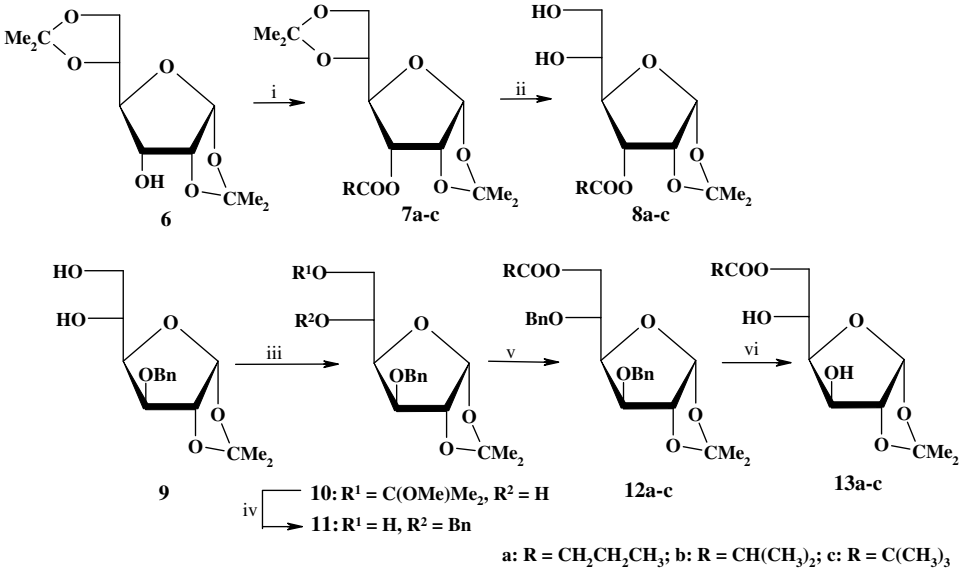


1,2-*O*-isopropylidene-D-hexofuranose functionality, and belonging the 3-*O*-acyl-D-allose (**8**) and 6-*O*-acyl-D-glucose (**13**) series.

The preparation of the 3-*O*-acyl-D-allose derivatives (**8**) was performed starting from the D-allose derivative **6**[5] (Scheme 1). Treatment of **6** with an excess of the pertinent acyl chloride in toluene in the presence of triethylamine, as previously reported for the preparation of the analogous D-glucose derivatives[3a] resulted in **8a–c** in almost quantitative yield. The hydrolytic removal of the 5,6-*O*-isopropylidene group of D-allose diacetonides **7a–c**, using 80% aqueous acetic acid, proved to be less selective than in the glucose series,[3a] resulting in the formation of appreciable amounts of by-products arising from complete removal of the acetal groups. This result is in accordance with the higher hydrolysis rate of the 1,2-*O*-isopropylidene functionality in the D-allose series with respect to the D-glucose series as pointed by Collins.[6] Satisfactory yields (85–88%) of the target derivatives **8a–c** were, however, obtained under milder conditions (40 °C) and with careful control of the reaction times.

Preliminary experiments aimed at generalizing the direct transformation of 3-*O*-acyl derivatives **1a–c** into 6-*O*-acyl ones (**13a–c**) through base catalyzed intramolecular acyl migration as successfully applied by Villa[7] to the 3-*O*-butyrate **1a**, led, in our hands, to the formation of inseparable mixtures of the two isomeric esters and were consequently abandoned. Also the method proposed by Frelek[8] for the preparation of the pivaloate **13c** by selective esterification at OH-6 of the easily available 1,2-*O*-isopropylidene-D-glucofuranose derivative, appeared not applicable to the preparation of the two other esters **13a**,**b** resulting, in preliminary assays, in complex mixtures of mono- and di-esterification products. With the objective of finding a general method for the preparation of 6-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives of type **13**, we turned our attention to the esterification at OH-6 of an intermediate 1,2-*O*-isopropylidene-D-glucofuranose derivative substituted at O-3 and O-5 with a removable protecting group. The preparation of the 3,5-di-*O*-benzyl derivative **11** by selective etherification of the known diol **9**[9] under phase transfer conditions[10] gave, in our hands, a complex mixture of several benzylation products. A satisfactory indirect selective etherification of the OH-5 of **9** was achieved through an effective sequence (Scheme 1) based on the transient formation of the mixed 1-methoxy-1-methylethyl acetal **10**, obtained by treatment of the diol



Scheme 1. Reagents and conditions: (i) RCOCl, Et_3N, PhMe, rt; (ii) 80% aq AcOH, 40 °C; (iii) 2-MP, $Et_3NH^+Cl^-$, CH_2Cl_2, rt; (iv) (a) BnBr, KOH, 18-crown-6, wet THF, (b) 10:1 MeOH–H_2O, AcOH traces; (v) RCOCl, 1:1 CH_2Cl_2–Py; (vi) H_2, 10% Pd/C, MeOH.

9 with 2-methoxypropene under triethylammonium chloride catalysis in dry dichloromethane. The use of a triethylammonium salt as mild acid, able to promote a fast addition of alcohols to 2-methoxypropene, but unable to give intramolecular transacetalations of another hydroxyl vicinal group has been previously reported.[11] In this case it is worthy of note that the addition is completely regioselective, resulting exclusively in the mixed acetal **10**, which was directly benzylated (BnBr, 18-crown-6/wet THF). The resulting protected intermediate was dissolved, without purification, in aqueous methanol acidified with acetic acid, to give, after chromatographic purification, alcohol **11** in an overall 75% yield from the diol **9**. Finally, the esterification of the alcohol **11** with the appropriate acyl chloride in a 1:1 CH_2Cl_2–pyridine mixture followed by catalytic debenzylation over palladium on charcoal led with good yields to the target 6-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives **13a**–**c**.

The human leukemia K562 cell line[12] was maintained in a humidified atmosphere of 5% CO_2/air in RPMI 1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; celbio, MI, Italy), 50 Units/mL penicillin and 50 μg/mL streptomycin.[13] In order to determine the ability of these compounds to inhibit cell growth and to induce erythroid differentiation, K562 cells were cultivated in the absence and in the presence of the indicated concentrations of compounds **8a**–**c** and **13a**–**c** and the cell number/mL determined with ZF Coulter Counter (Counter Electronics, Hialeah, FL, USA) at different days from the culture set-up, where the cells were seeded at 30,000 cells/mL. To determine possible effects on erythroid differentiation, the percent of benzidine-positive K562 cells was determined and compared to the induction obtained with the powerful inducer of erythroid differentiation cytosine arabinoside (ara-C).[14]

The results obtained using compounds **8a**–**c** and **13a**–**c** (Table 1) show the antiproliferative effects of the studied molecules, expressed as decrease in the proliferation efficiency of K562 cells. The 50% inhibition of cell growth (IC_{50}) was determined after 3 days of cell growth in the presence of a wide range of drugs concentrations.

Table 1. Inhibitory effects on K562 cell growth[a]

Compound	IC_{50}
1b	5.9 ± 1.1 mM
8a	3.1 ± 0.5 mM
8b	14.7 ± 1.7 mM
8c	6.1 ± 0.4 mM
13a	2.2 ± 0.1 mM
13b	5.2 ± 1.9 mM
13c	4.2 ± 1.3 mM
Ara-C	0.6 ± 0.05 μM

[a] The 50% inhibition of cell growth was determined after 3 days cultures in the presence of a wide range of drug concentrations.

From the data in Table 1, it appears that compounds **13a**–**c** are more active in inhibiting K562 cell growth than compounds **8a**–**c**. Among the compounds tested, compounds **8a** and **13a** were the most active in inhibiting cell growth. As far as the induction of differentiation is concerned, only compounds **8c** and **13c** stimulate erythroid differentiation to a level similar to those of the already reported[3a] compound **1b** (induction of differentiation being 28 ± 3.5). Interestingly, the active concentrations are not cytotoxic.

In conclusion, when the results of cell growth inhibition are compared, it is clear that 6-*O*-acyl-D-glucose derivatives (**8**) are more active than the 3-*O*-acyl-D-allose isomers (**13**). Furthermore, the highest inhibitory activities are displayed by compounds **8a** and **13a**, having the same acyl residue ($R = CH_2CH_2CH_3$). Inhibition of cell growth is not associated with high induction levels of erythroid differentiation, despite the fact that compounds with $R = C(CH_3)_3$ induce erythroid differentiation to an extent similar to that exhibited by compound **1b** and previously reported molecules.[3a] This result confirms our previous hypothesis[3c] that the biological activity of monosaccharide esters is probably related to the whole structure rather than to a possible release of the free fatty acids acting as the biologically active component.

1. Experimental

1.1. General methods

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 20 ± 2 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC 200 instrument at 200 and 50 MHz, respectively, in the appropriate solvent (internal standard Me_4Si). Assignments were made, when possible, with the help of DEPT experiments, for comparison with values for known compounds and applying the known[16] additivity rules. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254} with detection by UV light and/or with 10% phosphomolybdic acid in EtOH or H_2SO_4, and heating. Kieselgel 60 (E. Merck, 70–230 and 230–400 mesh, respectively) was used for column and flash chromatography. The solvents were dried and stored over 4 Å molecular sieves activated at least 24 h at 200 °C. $MgSO_4$ was used as the drying agent for solutions. Compounds **9**[9] and **6**[5] were prepared according to the published procedure.

1.2. 3-*O*-Butanoyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7a)

To a soln of **6**[5] (2.00 g, 7.68 mmol) in anhyd toluene (12.0 mL) and anhyd Et_3N (2.68 mL, 19.1 mmol), butyryl

chloride (2.4 mL, 23.0 mmol) was slowly added at 0 °C. The reaction mixture was warmed at room temperature until TLC analysis (1:1 hexane–EtOAc) showed the complete disappearance of the starting material (2 h), then it was filtered and concentrated to dryness under diminished pressure. A flash chromatography on silica gel (4:1 hexane–EtOAc) of the crude product afforded pure **7a** (2.43 g, 96%) as a white solid foam; R_f 0.19 (4:1 hexane–EtOAc); mp 42–43 °C; $[\alpha]_D$ +107.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.84 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.86 (m, 2H, H-2, H-3), 4.32 (ddd, 1H, $J_{4,5}$ 4.1 Hz, $J_{5,6a}$ 6.9 Hz, $J_{5,6b}$ 5.7 Hz, H-5), 4.16 (dd, 1H, $J_{3,4}$ 8.0 Hz, H-4), 4.04 (dd, 1H, $J_{6a,6b}$ 8.5 Hz, H-6a), 3.91 (dd, 1H, H-6b), 2.36 (t, 2H, *J* 7.7 Hz, CH_2CO), 1.67 (m, 2H, CH_3CH_2), 1.55, 1.42, 1.35, 1.33 [4s, each 3H, C(CH_3)$_2$], 0.97 (t, 3H, *J* 7.4 Hz, CH_3CH$_2$); ^{13}C NMR ($CDCl_3$): δ 172.7 (C=O), 109.9, 112.9 [2 × *C*(CH$_3$)$_2$], 104.1 (C-1), 77.6, 77.5 (C-2, C-3), 74.9 (C-5), 72.3 (C-4), 65.5 (C-6), 35.8 (*C*H$_2$CO), 26.6, 26.6, 26.2, 24.9 [2 × C(*C*H$_3$)$_2$], 18.3 (CH$_3$*C*H$_2$), 13.5 (*C*H$_3$CH$_2$). Anal. Calcd for $C_{16}H_{26}O_7$: C, 58.17; H, 7.93. Found: C, 58.31; H, 7.99.

1.3. 3-*O*-(2-Methylpropanoyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7b)

A soln of **6** (2.00 g, 7.68 mmol) was acylated with 2-methylpropanoyl chloride (2.4 mL, 23.0 mmol) by the procedure described above for **7a**. The reaction was complete in 2 h and a flash chromatography on silica gel (4:1 hexane–EtOAc) of the crude product afforded pure **7b** (2.46 g, 97%) as a white solid foam; R_f 0.20 (85:15 hexane–EtOAc); mp 30–31 °C; $[\alpha]_D$ +126.0 (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.84 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.83 (m, 2H, H-2, H-3), 4.33 (ddd, 1H, $J_{4,5}$ 4.1 Hz, $J_{5,6a}$ 6.9 Hz, $J_{5,6b}$ 5.8 Hz, H-5), 4.16 (dd, 1H, $J_{3,4}$ 8.2 Hz, H-4), 4.08 (dd, 1H, $J_{6a,6b}$ 8.5 Hz, H-6a), 3.91 (dd, 1H, H-6b), 2.63 [ept, 1H, *J* 7.0 Hz, C*H*(CH$_3$)$_2$], 1.54, 1.42, 1.35, 1.33 [4s, each 3H, C(CH_3)$_2$], 1.20 [d, 6H, *J* 7.0 Hz, CH(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$): δ 175.7 (C=O), 112.5, 109.6 [2 × *C*(CH$_3$)$_2$], 103.9 (C-1), 77.3, 77.2 (C-2, C-3), 74.7 (C-5), 72.1 (C-4), 65.3 (C-6), 33.4 (*C*HCO), 26.4, 26.3, 25.9, 24.7 [2 × C(*C*H$_3$)$_2$], 18.5 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{16}H_{26}O_7$: C, 58.17; H, 7.93. Found: C, 58.25; H, 8.01.

1.4. 3-*O*-(2,2-Dimethylpropanoyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7c)

A soln of **6** (2.00 g, 7.68 mmol) was acylated with 2,2-dimethylpropanoyl chloride (2.8 mL, 23.0 mmol) by the procedure described above for **7a**. The reaction was complete in 72 h and a flash chromatography on silica gel (85:15 hexane–EtOAc) of the crude product afforded pure **7c** (2.62 g, 99%) as a white solid foam; R_f 0.21 (85:15 hexane–EtOAc); mp 38–39 °C, lit.[15] mp 44–45 °C; $[\alpha]_D$ +115.6 (*c* 1.07, $CHCl_3$), lit.[15] $[\alpha]_D$ +113.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR data were identical to those reported;[15] ^{13}C NMR ($CDCl_3$): δ 177.5 (C=O), 112.7, 109.9 [2 × *C*(CH$_3$)$_2$], 104.2 (C-1), 77.6, 77.3 (C-2, C-3), 74.8 (C-5), 72.4 (C-4), 65.5 (C-6), 38.5 [*C*(CH$_3$)$_3$], 26.9, 26.8, 26.5, 26.2 [2 × C(*C*H$_3$)$_2$], 24.9 [C(*C*H$_3$)$_3$].

1.5. General procedure of selective hydrolysis of compounds 7a–c

A soln of the 3-*O*-acyl derivative (1 mmol) in aq 80% (v/v) AcOH (15 mL) was stirred at 40 °C. When TLC analysis (1:9 hexane–EtOAc) showed the complete disappearance of the starting material and formation of a slower moving product, the soln was immediately allowed to reach room temperature and was coevaporated three times with toluene (3 × 30 mL). The crude reaction product, purified by flash chromatography on silica gel with the appropriate eluant system, gave the desired pure diols **8a**–**c**.

1.5.1. 3-*O*-Butanoyl-1,2-*O*-isopropylidene-α-D-allofuranose (8a). From **7a** (1.23 g), syrup 918 mg (85%); R_f 0.24 (1:9 hexane–EtOAc); $[\alpha]_D$ +121.8 (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.82 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1), 4.86 (dd, 1H, $J_{2,3}$ 5.0 Hz, H-2), 4.85 (dd, 1H, $J_{3,4}$ 8.3 Hz, H-3), 4.15 (dd, 1H, $J_{4,5}$ 3.8 Hz, H-4), 3.98 (m, 1H, H-5), 3.62 (m, 2H, H-6a, H-6b), 3.08, 2.66 (2br s, 2H, 2 × OH), 2.37 (t, 2H, *J* 7.6 Hz, CH_2CO), 1.67 (m, 2H, CH_3CH_2), 1.54. 1.33 [2s, each 3H, C(CH_3)$_2$], 0.97 (t, 3H, *J* 7.3 Hz, CH_3CH$_2$); ^{13}C NMR ($CDCl_3$): δ 172.8 (C=O), 112.7 [*C*(CH$_3$)$_2$], 104.0 (C-1), 77.9, 77.4 (C-2, C-3), 71.3, 71.2 (C-4, C-5), 62.6 (C-6), 35.5 (*C*H$_2$CO), 26.6, 26.5 [C(*C*H$_3$)$_2$], 18.0 (CH$_3$*C*H$_2$), 13.3 (*C*H$_3$CH$_2$). Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.68; H, 7.64. Found: C, 53.75; H, 7.73.

1.5.2. 3-*O*-(2-Methylpropanoyl)-1,2-*O*-isopropylidene-α-D-allofuranose (8b). From **7b** (0.95 g), syrup 736 mg (88%); R_f 0.21 (1:4 hexane–EtOAc); $[\alpha]_D$ +126.0 (*c* 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.83 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.84 (m, 2H, H-2, H-3), 4.16 (m, 1H, H-4), 4.00 (m, 1H, H-5), 3.65 (m, 2H, H-6a, H-6b), 2.76 (br s, 1H, OH), 2.62 (ept, 1H, *J* 7.0 Hz, C*H*(CH$_3$)$_2$), 2.34 (br s, 1H, OH), 1.54, 1.33 [2s, each 3H, C(CH_3)$_2$], 1.20, 1.19 [2d, each 3H, *J* 7.0 Hz, CH(CH_3)$_2$]; ^{13}C NMR ($CDCl_3$): δ 176.1 (C=O), 112.7 [*C*(CH$_3$)$_2$], 104.1 (C-1), 78.0, 77.3 (C-2, C-3), 71.2, 71.1 (C-4, C-5), 62.6 (C-6), 33.4 (*C*HCO), 26.5, 26.4 [C(*C*H$_3$)$_2$], 18.6, 18.5 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.68; H, 7.64. Found: C, 53.79; H, 7.75.

1.5.3. 3-*O*-(2,2-Dimethylpropanoyl)-1,2-*O*-isopropylidene-α-D-allofuranose (8c). From **7c** (1.17 g), white solid foam 887 mg (86%); mp 62–64 °C; R_f 0.21 (3:7

hexane–EtOAc); $[\alpha]_D$ +137.3 (*c* 1.18, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.84 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.86 (dd, 1H, $J_{2,3}$ 5.2 Hz, H-2), 4.77 (dd, 1H, $J_{3,4}$ 8.6 Hz, H-3), 4.15 (dd, 1H, $J_{4,5}$ 3.4 Hz, H-4), 4.03 (m, 1H, H-5), 3.63–3.53 (m, 2H, H-6a, H-6b), 2.91, 2.51 (2br s, 2H, 2 × OH), 1.52, 1.32 [2s, each 3H, $C(CH_3)_2$], 1.23 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$): δ 177.7 (C=O), 112.9 [$C(CH_3)_2$], 104.4 (C-1), 78.2, 77.3 (C-2, C-3), 71.3, 71.1 (C-4, C-5), 62.7 (C-6), 38.6 [$C(CH_3)_3$], 26.8, 26.6 [$C(CH_3)_2$], 27.0 [$C(CH_3)_3$]. Anal. Calcd for $C_{14}H_{24}O_7$: C, 55.25; H, 7.95. Found: C, 55.37; H, 8.02.

1.6. 3-*O*-Benzyl-6-*O*-(1-methoxy-1-methylethyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (10)

A soln of **9**[9] (5.70 g, 18.3 mmol) and $Et_3N{\cdot}HCl$ (1.36 g, 9.86 mmol) in dry CH_2Cl_2 (260 mL) was cooled to 0 °C under argon, then a soln of freshly distilled 2-methoxypropene (3 mL, 30.0 mmol) in dry CH_2Cl_2 (27 mL) was added dropwise. The mixture was gently warmed at room temperature and left with stirring until **9** was completely consumed (4 h, 1:1 hexane–EtOAc). The mixture was neutralized by addition of 60 mL satd aq $NaHCO_3$ and stirred for 15 min at room temperature. The aq phase was extracted with CH_2Cl_2 (4 × 30 mL) and the combined extracts were collected and dried ($MgSO_4$). The combined organic phases were concentrated to give a crude product (6.52 g) containing (^1H and ^{13}C NMR) exclusively **10**. A flash chromatography on silica gel (3:2 hexane–EtOAc + 0.1% of Et_3N) of a sample of the crude reaction product yielded pure **10** as a syrup; R_f 0.50 (1:1 hexane–EtOAc); $[\alpha]_D$ −2.8 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38–7.27 (m, 5H, aromatic H), 5.91 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.73 and 4.61 (AB system, 2H, $J_{A,B}$ 11.7 Hz, CH_2Ph), 4.61 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.18–4.05 (m, 3H, H-3, H-4, H-5), 3.66 (dd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b}$ 9.8 Hz, H-6a), 3.51 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 3.21 (s, 3H, OCH_3), 2.69 (d, 1H, $J_{5,OH}$ 5.1 Hz, OH-5), 1.48, 1.32 [2s, each 3H, $(CCH_3)_2$]; 1.35 [s, 6H, $(CH_3)_2C$–OCH_3]; ^{13}C NMR ($CDCl_3$): δ 137.4 (aromatic C), 128.5–127.8 (aromatic CH), 111.6 [$C(CH_3)_2$], 105.1 (C-1), 100.1 [$(CH_3)_2C$–OCH_3], 82.2, 81.9 (C-2, C-3), 79.7 (C-4), 72.2 (CH_2Ph), 68.0 (C-5), 62.7 (C-6), 48.5 (OCH_3), 26.7, 26.2 [$(CH_3)_2C$], 24.4, 24.4 [$(CH_3)_2C$–OCH_3].

1.7. 3,5-di-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (11)

To a soln of crude **10** (11.3 g) in wet THF (145 mL), powdered KOH (6.64 g, 118.6 mmol) and 18-crown-6 (99 mg, 0.38 mmol) were added. The mixture was vigorously stirred for 30 min at room temperature, then benzyl bromide (7.5 mL, 59.3 mmol) was added and left to react at the same temperature. When TLC analysis (1:1 hexane–EtOAc) revealed the formation of a single product (24 h, R_f 0.71), MeOH (5 mL) was added and the stirring prolonged for 10 min. Solvents were evaporated under diminished pressure and the residue was partitioned between water (80 mL) and CH_2Cl_2 (200 mL). The aq phase was further extracted with CH_2Cl_2 (4 × 50 mL). The organic extracts were dried ($MgSO_4$), filtered, and concentrated under diminished pressure. The crude syrup was dissolved in 10:1 MeOH–water (100 mL) containing AcOH (1 mL) and the reaction mixture was stirred at room temperature. After 3 h TLC analysis (1:1 hexane–EtOAc) revealed the complete disappearance of the starting material and formation of a slower moving product (R_f 0.42). The soln was neutralized by the addition of Et_3N (2.0 mL), evaporated under diminished pressure, and coevaporated with toluene (5 × 30 mL). A flash chromatography on silica gel (1:1 hexane–EtOAc) of the crude residue afforded pure **11** (8.93 g, 75% from diol **9**) as a colorless syrup; R_f 0.42 (1:1 hexane–EtOAc); $[\alpha]_D$ −23.5 (*c* 1.1, $CHCl_3$), lit.[10] $[\alpha]_D$ −21.8 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.32–7.24 (m, 10H, aromatic H), 5.91 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.63 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.67 and 4.47 (AB system, 2H, $J_{A,B}$ 12.5 Hz, CH_2Ph), 4.63 and 4.47 (AB system, 2H, $J_{A,B}$ 11.4 Hz, CH_2Ph), 4.30 (dd, 1H, $J_{3,4}$ 3.1 Hz, $J_{4,5}$ 8.3 Hz, H-4), 4.10 (d, 1H, H-3), 4.06–3.89 (m, 2H, H-5, H-6a), 3.79 (dd, 1H, $J_{6a,6b}$ 13.0 Hz, $J_{5,6b}$ 5.1 Hz, H-6b), 1.49, 1.32 (2s, each 3H, $(CH_3)_2C$); ^{13}C NMR data were identical to those reported.[10]

1.8. 6-*O*-Butanoyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (12a)

To a soln of alcohol **11** (2.16 g, 5.40 mmol) in dry 1:1 CH_2Cl_2–Py (24 mL), butanoyl chloride (1.73 mL, 16.2 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature until TLC analysis (1:1 hexane–EtOAc) showed the complete disappearance of the starting material (1 h), then solvents were evaporated under diminished pressure and coevaporated with toluene (4 × 10 mL). The residue was partitioned between water (20 mL) and CH_2Cl_2 (30 mL), the phases were separated and the aq phase further extracted with CH_2Cl_2 (4 × 20 mL). The collected organic extracts were dried ($MgSO_4$), filtered, concentrated under diminished pressure, and the crude product was subjected to flash chromatography on silica gel (4:1 hexane–EtOAc) to afford pure **11** (1.96 g, 77%) as a colorless syrup; R_f 0.64 (1:1 hexane–EtOAc); $[\alpha]_D$ −35.4 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.31–7.22 (m, 10H, aromatic H), 5.90 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.61 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.69 and 4.48 (AB system, 2H, $J_{A,B}$ 11.5 Hz, CH_2Ph), 4.46 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.66 and 4.42 (AB system, 2H, $J_{A,B}$ 11.2 Hz, CH_2Ph), 4.30 (dd, 1H, $J_{4,5}$ 9.1 Hz, H-4), 4.14 (dd, 1H, $J_{6a,6b}$ 11.5 Hz, $J_{5,6a}$ 4.6 Hz, H-6a), 4.06 (dd, 1H, $J_{5,6b}$ 2.3 Hz,

H-6b), 4.04 (ddd, 1H, H-5), 2.31 (t, 2H, *J* 7.3 Hz, C*H*$_2$CO), 1.65 (m, 2H, CH$_3$C*H*$_2$), 1.48, 1.31 (2s, each 3H, C(C*H*$_3$)$_2$), 0.93 (t, 3H, *J* 7.3 Hz, C*H*$_3$CH$_2$); ^{13}C NMR ($CDCl_3$): δ 173.4 (C=O), 138.1, 137.4 (aromatic C), 128.5–127.6 (aromatic CH), 111.8 [*C*(CH$_3$)$_2$], 105.1 (C-1), 81.7, 81.6 (C-2, C-3), 78.7 (C-4), 74.2 (C-5), 72.3, 72.0 (*C*H$_2$Ph), 63.3 (C-6), 36.2 (*C*H$_2$CO), 26.8, 26.3 [C(*C*H$_3$)$_2$], 18.4 (CH$_3$*C*H$_2$), 13.6 (*C*H$_3$CH$_2$). Anal. Calcd for $C_{27}H_{34}O_7$: C, 68.92; H, 7.28. Found: C, 68.80; H, 7.10.

1.9. 6-*O*-(2-Methylpropanoyl)-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (12b)

A soln of **11** (2.11 g, 5.27 mmol) was acylated with 2-methylpropanoyl chloride (1.64 mL, 15.8 mmol) by the procedure described above for **12a**. The reaction was complete in 1 h and a flash chromatography on silica gel (4:1 hexane–EtOAc) of the crude product afforded pure **12b** (2.44 g, 98%) as a white solid foam; R_f 0.64 (1:1 hexane–EtOAc); mp 39–41 °C; $[\alpha]_D$ −34.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33–7.19 (m, 10H, aromatic H), 5.91 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.61 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.66 and 4.48 (AB system, 2H, $J_{A,B}$ 11.5 Hz, C*H*$_2$Ph), 4.45 (d, 1H, $J_{3,4}$ 3.1 Hz, H-3), 4.71 and 4.40 (AB system, 2H, $J_{A,B}$ 11.1 Hz, C*H*$_2$Ph), 4.31 (dd, 1H, $J_{4,5}$ 9.0 Hz, H-4), 4.14 (dd, 1H, $J_{6a,6b}$ 9.1 Hz, $J_{5,6a}$ 1.6 Hz, H-6a), 4.09 (dd, 1H, $J_{5,6b}$ 4.4 Hz, H-6b), 4.03 (ddd, 1H, H-5), 2.30 [ept, 1H, *J* 7.0 Hz, C*H*(CH$_3$)$_2$], 1.48, 1.31 [2s, each 3H, C(C*H*$_3$)$_2$], 1.17, 1.16 [2d, each 3H, *J* 7.0 Hz, (C*H*$_3$)$_2$CH]; ^{13}C NMR ($CDCl_3$): δ 176.0 (C=O), 138.1, 137.4 (aromatic C), 128.4–127.5 (aromatic CH), 111.8 [*C*(CH$_3$)$_2$], 105.0 (C-1), 81.7, 81.5 (C-2, C-3), 78.6 (C-4), 74.2 (C-5), 71.9, 72.2 (*C*H$_2$Ph), 63.3 (C-6), 34.0 (*C*HCO), 26.7, 26.3 [C(*C*H$_3$)$_2$], 18.9 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{27}H_{34}O_7$: C, 68.92; H, 7.28. Found: C, 68.77; H, 7.08.

1.10. 6-*O*-(2,2-Dimethylpropanoyl)-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (12c)

A soln of **11** (2.00 g, 5.00 mmol) was acylated with 2-methylpropanoyl chloride (1.84 mL, 15.2 mmol) by the procedure described above for **12a**. The reaction was complete in 1 h and a flash chromatography on silica gel (4:1 hexane–EtOAc) of the crude product afforded pure **12b** (2.36 g, 97%) as colorless syrup; R_f 0.54 (3:2 hexane–EtOAc); $[\alpha]_D$ −36.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.31–7.24 (m, 10H, aromatic H), 5.90 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.60 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.64 and 4.49 (AB system, 2H, $J_{A,B}$ 11.6 Hz, C*H*$_2$Ph), 4.44 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 4.72 and 4.40 (AB system, 2H, $J_{A,B}$ 11.2 Hz, CH$_2$Ph), 4.31 (dd, 1H, $J_{4,5}$ 9.0 Hz, H-4), 4.20 (m, 2H, H-6a, H-6b), 4.05 (ddd, 1H, $J_{5,6b}$ 4.4 Hz, $J_{5,6a}$ 1.9 Hz, H-5), 1.47, 1.31 [2s, each 3H, C(C*H*$_3$)$_2$], 1.21 [s, 9H, *J* 7.0 Hz, C(C*H*$_3$)$_3$]; ^{13}C NMR ($CDCl_3$): δ 178.3 (C=O), 138.2, 137.5 (aromatic C), 128.5–127.6 (aromatic CH), 111.8 [*C*(CH$_3$)$_2$], 105.1 (C-1), 81.8, 81.6 (C-2, C-3), 78.6 (C-4), 74.3 (C-5), 72.1 (2 ×*C*H$_2$Ph), 63.3 (C-6), 38.9 [*C*(CH$_3$)$_3$], 27.2 [C(*C*H$_3$)$_3$], 27.8, 26.4 [C(*C*H$_3$)$_2$]. Anal. Calcd for $C_{28}H_{36}O_7$: C, 69.04; H, 7.49. Found: C, 69.16; H, 7.57.

1.11. General procedure of debenzylation of 12a–c

A soln of 6-*O*-acyl derivatives (1.00 mmol) in MeOH (50 mL) containing 10% Pd on charcoal (170 mg) was stirred at room temperature under an H_2 atmosphere until TLC analysis (3:7 hexane–EtOAc) showed the complete disappearance of the starting material (24 h). The suspension was filtered over Celite and concentrated under diminished pressure. Crystallization of the crude residue (hexane–EtOAc) afforded desired diols **13a**–**c**.

1.11.1. 6-*O*-Butanoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (13a). From **12a** (1.00 g), white solid foam 569 mg (92%); R_f 0.33 (3:7 hexane–EtOAc); mp 83–84 °C, lit.[7b] mp 85 °C; $[\alpha]_D$ −1.3 (*c* 0.9, $CHCl_3$), lit.[7b] $[\alpha]_D$ −1.1 (*c* 1.1, $CHCl_3$). The NMR data were in agreement with those reported in literature.[7b]

1.11.2. 6-*O*-(2-Methylpropanoyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (13b). From **12b** (1.44 g), white solid 773 mg (81%); R_f 0.27 (3:7 hexane–EtOAc); mp 107–109 °C; $[\alpha]_D$ −2.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.97 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.54 (d, 1H, $J_{2,3}$ 0 Hz, H-2), 4.44 (m, 1H, H-5), 4.38 (d, 1H, $J_{3,4}$ 2.7 Hz, H-3), 4.24 (m, 2H, H-6a, H-6b), 4.09 (dd, 1H, $J_{4,5}$ 5.9 Hz, H-4), 2.62 [ept, *J* 7.0 Hz, 1H, C*H*(CH$_3$)$_2$], 1.49, 1.33 [2s, each 6H, C(C*H*$_3$)$_2$], 1.20 [d, 6H, *J* 7.0 Hz, CH(C*H*$_3$)$_2$]; ^{13}C NMR ($CDCl_3$): δ 174.3 (C=O), 111.8 [*C*(CH$_3$)$_2$], 104.9 (C-1), 85.2 (C-2), 79.2 (C-4), 75.6 (C-3), 69.4 (C-5), 66.0 (C-6), 34.0 (*C*HCO), 26.8, 26.2 [C(*C*H$_3$)$_2$], 18.9 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.78; H, 7.64. Found: C, 53.70; H, 7.59.

1.11.3. 6-*O*-(2,2-Dimethylpropanoyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (13c). From **12c** (1.18 g), white solid 599 mg (81%); R_f 0.33 (1:1 hexane–EtOAc); mp 148–149 °C, lit.[8] mp 146–147 °C; $[\alpha]_D$ −5.5 (*c* 1.1, $CHCl_3$), lit.[8] $[\alpha]_D$ −5.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR data were identical to those reported;[8] ^{13}C NMR ($CDCl_3$): δ 179.4 (C=O), 111.8 [*C*(CH$_3$)$_2$], 104.9 (C-1), 85.0 (C-2), 79.1 (C-4), 75.4 (C-3), 69.0 (C-5), 66.1 (C-6), 38.9 [*C*(CH$_3$)$_3$], 27.1 [C(*C*H$_3$)$_3$], 27.7, 26.2 [C(*C*H$_3$)$_2$].

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