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Preparation and Evaluation of the In Vitro Erythroid Differentiation Induction Properties of Some Esters of Methyl 3,4-*O*-Isopropylidene-β-D-galactopyranoside and 2,3-*O*-Isopropylidene-D-mannofuranose

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Abstract—Two series of glycide esters of short fatty acids, designed for avoiding intramolecular transesterification, were prepared and tested for in vitro erythroid differentiation induction activities using the K562 cell line as experimental system. The 6-*O*-isobutiryl and pivaloyl derivatives of methyl 3,4-*O*-isopropylidene-β-D-galactopyranosides as well the same 1-*O*-esters of 2,3-*O*-isopropylidene-α- and β-D-mannofuranose exhibit biological activities much higher that the corresponding acids and could be proposed as possible agents to modulate production of embryo-fetal hemoglobins by human erythroid cells.

Introduction

Pharmacologically-mediated regulation of the expression of human γ-globin genes has been suggested in the search of potential therapeutic agents in hematological disorders, since increases in fetal Hb levels can be clinically beneficial in sickle cell disease[1] and β-thalassemia.[2] Therefore, a number of studies have been performed to identify compounds able to stimulate γ-globin gene expression.[3] Among possible biological response modifiers, compounds of great interest are short fatty acids.[4,5] Accordingly, butyrates have been used in therapeutic trials to stimulate fetal hemoglobin gene expression in patients with hemoglobinopathies.[6,7]

The K562 cell line, isolated and characterized by Lozzio and Lozzio[8] from a patient with chronic myelogenous leukemia in blast crisis, has been proposed as a very useful in vitro model system (a) to study the molecular mechanism(s) regulating the expression of embryonic and fetal human globin genes,[9] as well as (b) to determine the therapeutic potential of new differentiating compounds.[10] K562 cells exhibit a low proportion of hemoglobin-synthetizing cells under standard cell growth conditions, but are capable to undergo erythroid differentiation when treated with a variety of compounds, including hemin, cytosine arabinoside (ara-C), butyric acid, 5-azacytidine, mithramycin, and chromomycin, cisplatin and cisplatin analogues, tallimustine.[9–15] Following erythroid induction of K562 cells, a sharp increase of cytoplasmic accumulation of Hb Portland ($\zeta_2\gamma_2$) and Hb Gower 1 ($\zeta_2\varepsilon_2$) is observed, accompanied by an increase in the expression of human ε and γ globin genes.[11–15]

Interestingly, erythroid inducers of K562 cells, for instance hydroxyurea, butyric acid, hemin and tallimustine, were found to be able to stimulate the increase HbF production by normal erythroid precursor cells.[16–20] Therefore, K562 cells represent an experimental system for a first screening of compounds of possible interest in the pharmacological treatment of hemoglobinathies, including β-thalassemia.[15]

We have recently reported[21] the synthesis of 12 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives,

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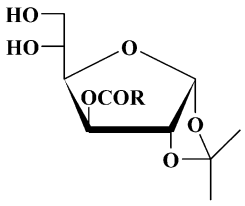
and tested their effects in augmenting the proportion of benzidine-positive (hemoglobin-containing) cells in treated K562 cell populations. The results obtained demonstrated that two of the newly synthesised compounds were potent inducers of erythroid differentiation of K562 cells. In addition, these same compounds potentiate erythroid induction of K562 cells treated with sub-optimal concentrations of cytosine arabinoside (ara-C), retinoic acid and mithramycin.[21] Table 1 shows a summary of the results obtained with the compounds **1a**, **1b** and **1c**. Compounds **1b** and **1c** were considered of interest for further experiments aimed at the induction of γ-globin gene expression.[21]



1a : R = $CH_2CH_2CH_3$
1b : R = $CH(CH_3)_2$
1c : R = $C(CH_3)_3$

Table 1. Effects of 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives on in vitro growth and erythroid differentiation of human leukemic K562 cells

Compound	Cell proliferation IC_{50} (mM)	Erythroid induction[a] % of benzidine-positive cells (mM)
1a	0.8	25.4±3.8 (2 mM)
1b	2.5	54.2±8.2 (4 mM)
1c	1.5	52.8±2.9 (3 mM)

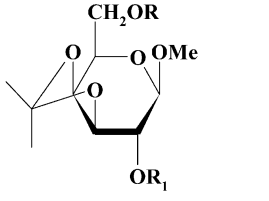
[a]Results are presented as maximum level of % of benzidine-positive (hemoglobin-containing) cells ±SD, reached within a 9 days induction period at the indicated concentrations of tested compounds.

Unfortunately, a rapid intramolecular acyl shift from O-3 to O-6 was observed both under chemical[22] and biological[23] catalysis for **1b**. A similar behaviour was previously reported by Villa et al.[24] for **1a**. In order to circumvent this unwanted modification of our α-branched glycide esters, we have now prepared some other derivatives that, owing to the unfavourable relative orientation of the acyl and the free hydroxyl groups, are expected to be not subjected to intramolecular transesterification. We present in this paper the preparation of some esters of the D-galactopyranoside and the D-mannofuranose series, and their biological activity as erythroid differentiation agents on human K562 cells.

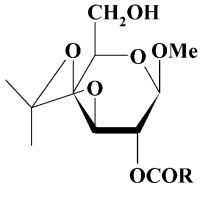
Chemistry

The preparation of methyl 2-*O*-acyl-3,4-*O*-isopropylidene-β-D-galactopyranosides (**4**) was achieved from the mixed diacetal **2**[25] employing a one pot procedure involving the esterification of **2** with the appropriate acyl chloride in toluene and trietylamine, followed by removal of the acyclic acetal function with pyridinium tosylate in methanol. Analytically pure compounds **4a–c** were thus obtained with satisfactory yield (53–65%) after flash chromatography of the crude reaction mixtures followed by crystallization. The 6-*O*-acyl isomers **5a–c** were obtained from the benzyl ether **3**,[26] that was routinely 6-*O*-acylated and debenzylated by hydrogenolysis with 10% $Pd(OH)_2$ on charcoal in ethyl acetate. Compounds **5a–c** were finally obtained in excellent overall yields (94–96%) by flash chromatography.

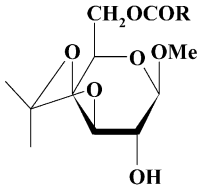
The preparation of the 1-*O*-acyl-2,3-*O*-isopropylidene-α- and-β-D-mannofuranose derivatives (**8** and **9**) was performed, with minor modifications, following the procedure described by Baldwin et al.[24a] for **8a**. The composition of the anomeric ester mixtures obtained by



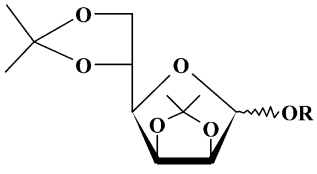
2: R = $C(OMe)Me_2$, R_1 = H
3: R = H, R_1 = Bn



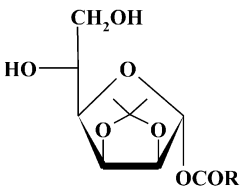
4a: R = $CH_2CH_2CH_3$
4b: R = $CH(CH_3)_2$
4c: R = $C(CH_3)_3$



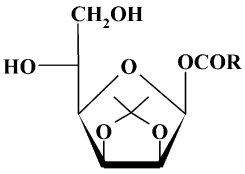
5a: R = $CH_2CH_2CH_3$
5b: R = $CH(CH_3)_2$
5c: R = $C(CH_3)_3$



6: R = H
7a: R = $COCH_2CH_2CH_3$
7b: R = $COCH(CH_3)_2$
7c: R = $COC(CH_3)_3$



8a: R = $CH_2CH_2CH_3$
8b: R = $CH(CH_3)_2$
8c: R = $C(CH_3)_3$



9a: R = $CH_2CH_2CH_3$
9b: R = $CH(CH_3)_2$
9c: R = $C(CH_3)_3$

treatment of commercial **6** (Sigma) with the appropriate acyl chloride, was esthablished by ^{1}H NMR analysis on the basis of the integration of the signals of the anomeric protons (see Experimental). The reaction of **6** with butanoyl chloride led, in agreement with the results by Baldwin et al.,[24a] to a larger amount of α anomer (α/β ratio >9:1), while with 2-methylpropanoyl chloride the proportion of the α anomer decreased (α/β ratio 3:1), and, finally, with 2,2-dimethylpropanoyl chloride an inverted prevalence of the β anomer was found (α/β ratio 4:6). The selective hydrolysis of the 5,6-*O*-isopropylidene group of derivatives **7** was performed with $CuCl_2$ in ethanol, as previously proposed by Iwata and Ohrui.[27] In the case of the α anomers, α-**7a**–**c**, the rate of the hydrolysis was rather slow, requiring about 16 h for the complete disappearance of the starting products, and the selectivity of the process was good giving the partially deprotected products **8a**–**c** in high yields (about 80%). Conversely, the β anomers reacted faster (about 4 h) but the yields of the desired 2,3-monoisopropylidene derivatives **9b**,**c** were rather low (about 40%), some lower moving products, probably the completely deisopropylidenated derivatives, being formed.

Biological Studies

Human myeloid leukemia K562(S) cells[10] were maintained in RPMI 1640 (Gibco, BRL, Milan, Italy) in 10% fetal bovine serum (Gibco, BRL, Milan, Italy), 5% CO_2 supplemented with 50 units/mL penicillin, 50 μg/mL streptomycin.[11] Cell growth was studied by determining the cell number/mL after different days of in vitro cell cultures.[11] In order to study the ability of the compounds to induce erythroid differentiation, K562 cells were cultured in the absence or in the presence of the indicated concentrations of the studied esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose, and most of the corresponding fatty acids, ara-C, tallimustine and mithramycin. The percent of benzidine-positive K562 cells during the induction was determined daily in order to quantify the percent of hemoglobin-containing cells.[15] As clearly evident, from the results presented in Table 2, most of the compounds (for instance **5b**, **5c**, **8b**, **8c**, **9c**) showed capacity to induce erythroid differentiation (percent of benzidine positive cells higher than 40%) of K562 cells, although to a lower level when compared to ara-C,[12] tallimustine[15] and mithramycin[13] (Table 2) as well as other inducing compounds, including cisplatin and cisplatin analogues[14] (data not shown). By contrast, other tested compounds (for instance, **4a**–**c**, **5a**, **8a**, **9b**) showed lower activity. Interestingly, in agreement with our previous observations[21] compounds with $R=CH(CH_3)_2$ and $R=C(CH_3)_3$ exhibited high activity in inducing erythroid differentiation of K562 cells. In addition, Table 2 shows also that most of the newly synthetized esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose cause a decrease in the proliferation efficiency of K562 cells. 50% inhibition of cell growth (IC_{50}) occurs when K562 cells are cultured for 4 days in the presence of drugs concentrations ranging from 5 mM (**5b**) to 1.5 mM (**9c**). This phenomenon is expected, since it is known that induction of erythroid differentiation of K562 cells is associated with a decrease of cell growth rates of differentiated cells.[11] The induction ability of compounds **5b**, **5c**, **8b**, **8c**, **9c** is significantly higher than that exhibited by isobutyric and pivalic acid.

Table 2. Effects of esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose on in vitro growth and erythroid differentiation of human leukemic K562 cells

Compound	Cell proliferation IC_{50}	Erythroid induction[a] % of benzidine-positive cells (mM, nM)
4a	4 mM	24.4±2.1 (6 mM)
4b	4 mM	20.3±1.4 (3 mM)
4c	4 mM	14.6±1.8 (4 mM)
5a	3 mM	32.6±2.3 (6 mM)
5b	5 mM	43.5±5.7 (6 mM)
5c	2.5 mM	42.5±3.5 (4 mM)
8a	2 mM	30.6±1.7 (1 mM)
8b	4 mM	57.3±4.9 (6 mM)
8c	2 mM	48±8.6 (4 mM)
9b	3 mM	29.4±9.1 (3 mM)
9c	1.5 mM	49.33±7.9 (2 mM)
Butyric acid	2.4 mM	35.3±3.7 (3 mM)
Isobutyric acid	4.8 mM	14±2.7 (5 mM)
Pivalic acid	4.5 mM	12±1.7 (5 mM)
Mithramycin	10 nM	91.5±5.1 (20 nM)
ara-C	40 nM	78.3±4.1 (500 nM)
Tallimustine	15 mM	85.7±3.4 (50 nM)

[a]Results are presented as maximum level of % of benzidine-positive (hemoglobin-containing) cells ±SD reached within a 9 days induction period at the indicated concentrations of tested compounds.

Table 3. Synergism between ara-C and esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose on in vitro growth and erythroid differentiation of human leukemic K562 cells

Compound	Concentration	Erythroid induction[a] % of benzidine-positive cells
4a	2 mM	34.2±3.5
4b	3 mM	30.3±4.1
4c	2 mM	24.4±4.5
5a	2 mM	35.1±3.3
5b	6 mM	42.6±4.8
5c	4 mM	36±1.4
8a	1 mM	83.7±3.2
8b	5.5 mM	69.5±6.6
8c	3.5 mM	60.2±3.1
9b	3 mM	82.2±5.2
9c	1.5 mM	56±4.6
ara-C	200 nM	28.2±2.1

[a]Results are presented as maximum level of % of benzidine-positive (hemoglobin-containing) cells ±SD reached within a 9 days induction period at the indicated concentrations of tested compounds. Suboptimal concentrations of ara-C (200 nM) were used in combination with the tested compounds.

A second general feature of erythroid inducing agents is related to the possibility to act in synergism with other compounds. This could be of interest for the design of agents useful for efficient combined therapy. Table 3 shows that when sub-optimal concentrations of ara-C are administered to K562 cells together with the tested compounds, high induction of K562 erythroid differentiation was in some cases obtained. This was particularly evident for compounds **8a** and **9b**. These data suggest that these compounds could potentiate the effects of other known inducers of K562 cells erythroid differentiation.

Conclusions

The data presented in this paper demonstrate that some of the studied esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose (**5b**, **5c**, **8b**, **8c**, and **9c**; see Table 2) could be considered as inducers of K562 cell erythroid differentiation exhibiting a biological activity much higher than that of the corresponding isobutyric and pivalic acid. The studied compounds are expected to be not subjected to intramolecular transesterification, unlike 3-*O*-acyl-1,2-*O*-isopropylidene-D-glucofuranose derivatives.[21] In addition, we like to underline that some of the compounds tested (**8a** and **9b**; see Table 3) exhibit capability to act in synergism with suboptimal concentrations of ara-C in inducing erythroid differentiation.

Our results do not explain the mechanism of action of the tested compounds; in order to study this specific, but very important, point further experiments should be undertaken, including analysis of the uptake of the compounds,[28] gene expression profile of treated cells,[29] alteration of erythroid-specific markers during induction.[30] We hypothesise, however, that the set of biological functions altered by compound **8b** and **9a** could be different to those regulated following treatment with ara-C. We found indeed that a combined treatment (**8b** plus ara-C or **9a** plus ara-C) cause a synergistic effect on erythroid induction.

In any case, the results presented in this paper suggest that some of the esters of methyl 3,4-*O*-isopropylidene-β-D-galactopyranoside and 2,3-*O*-isopropylidene-D-mannofuranose we have studied in the present paper could be proposed as possible agents to modulate production of embryo-fetal hemoglobins by human erythroid cells. In this respect, these compounds are candidates to determine whether they stimulate HbF production in normal erythroid precursors, as already observed for many erythroid inducers of K562 cells.[15–20]

Experimental

General chemical methods were previously reported by some of us[26]. 2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose (**6**) (Sigma) was dried at 50 °C and 0.1 torr for 5 h. Previously described procedures were employed for the preparation of methyl 3,4-*O*-isopropylidene-6-*O*-(1-methoxy-1-methylethyl)-β-D-galactopyranoside (**2**)[25] and methyl 2-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**3**).[26]

Methyl 2-*O*-acyl-3,4-*O*-isopropylidene-β-D-galactopyranosides (4a–c). To a solution of **2**[25] (919 mg, 3.0 mmol) in anhydrous toluene (12 mL), anhydrous Et_3N (1.0 mL, 7.2 mmol) and the required acyl chloride (6.6 mmol) were added at room temperature. The reaction mixture was stirred at room temperature until TLC analysis showed the complete disappearance of the starting material, then it was filtered and concentrated to dryness in vacuo. The crude product was dissolved in MeOH (18 mL) containing pyridinium tosylate (18 mg, 0.11 mmol), stirred at room temperature for 30 min, neutralized by addition of Na_2CO_3, stirred for 15 min at room temperature, filtered and concentrated under reduced pressure. The crude reaction product, purified by flash chromatography, gave the desired 2-*O*-acyl derivatives **4a**–**c**.

Methyl 2-*O*-butanoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (4a). White solid, 53% yield, R_f 0.29 (4:6 hexane–EtOAc); mp 79–81 °C (from EtOAc–hexane); $[\alpha]_D$ +20.1 (*c* 5.0, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 0.96 (t, 3H, $J = 7.4$ Hz, CH_3CH_2); 1.34, and 1.56 (2 s, each 3H, $C(CH_3)_2$); 1.68 (m, 2H, CH_3CH_2); 2.35 (t, 2H, $J = 7.3$ Hz, CH_2CO); 2.58 (bs, 1H, OH); 3.49 (s, 3H, OCH_3); 3.84–4.01 (m, 3H, H-5, H-6, and H-6′); 4.20 (m, 2H, H-3, and H-4); 4.28 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 4.98 (m, 1H, H-2). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.25%; H, 7.95%. Found C, 55.11%; H, 8.02%.

Methyl 2-*O*-(2-methylpropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (4b). White solid, 65% yield, R_f 0.25 (1:1 petroleum ether–EtOAc); mp 74–75 °C (from hexane); $[\alpha]_D$ + 17.4 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.19 (d, 6H, $J = 7.0$ Hz, $(CH_3)_2CH$); 1.34, and 1.56 (2 s, each 3H, $C(CH_3)_2$); 2.31 (s, 1H, OH); 2.59 (e, 1H, *CH*CO); 3.49 (s, 3H, OCH_3), 3.83–4.02 (m, 3H, H-5, H-6, and H-6′); 4.20 (m, 2H, H-3, and H-4); 4.29 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.96 (m, 1H, H-2). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.25%; H, 7.95%. Found C, 55.43%; H, 7.84%.

Methyl 2-*O*-(2,2-dimethylpropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (4c). White solid, 58% yield, R_f 0.25 (1:1 hexane–EtOAc); mp 85–87 °C (from petroleum ether); $[\alpha]_D$ +19.3 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$), δ 1.22 (s, 9H, $(CH_3)_3C$); 1.34 and 1.56 (2 s, each 3H, $C(CH_3)_2$); 2.33 (s, 1H, OH); 3.49 (s, 3H, OCH_3); 3.83–4.02 (m, 3H, H-5, H-6 and H-6′); 4.20 (m, 2H, H-3, and H-4); 4.28 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 4.95 (m, 1H, H-2). Anal. calcd for $C_{15}H_{26}O_7$: C, 56.59%; H, 8.23%. Found C, 56.69%; H, 8.38%.

Methyl 6-*O*-acyl-3,4-*O*-isopropylidene-β-D-galactopyranosides (5a–c). To a solution of **3**[26] (648 mg, 2.0 mmol) in anhydrous toluene (12 mL), anhydrous Et_3N (0.68 mL, 4.8 mmol) and the required acyl chloride (4.4 mmol) were added at room temperature. The reaction mixture was stirred at room temperature until TLC analysis (3:7 hexane–EtOAc) showed the complete dis-

appearance of the starting material, then it was filtered and concentrated to dryness in vacuo. The crude product was dissolved in EtOAc (16 mL) containing 10% $Pd(OH)_2$ on charcoal (160 mg), stirred at room temperature under H_2 for 2–5 h until the starting material disappeared (TLC analysis). The suspension was filtered over Celite, concentrated under reduced pressure and the residue purified by flash chromatography to give the desired 6-*O*-acyl derivatives **5a–c**.

Methyl 6-*O*-butanoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (5a). White solid, 93% yield, R_f 0.32 (1:1 hexane–EtOAc); mp 76–78 °C (from EtOAc–hexane); $[\alpha]_D$ +18.8 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 0.96 (t, 3H, $J = 7.4$ Hz, CH_3–CH_2); 1.34 and 1.52 (2 s, each 3H, $C(CH_3)_2$); 1.67 (m, 2H, CH_3CH_2); 2.34 (t, 2H, $J = 7.3$ Hz, CH_2CO); 2.76 (d, 1H, $J_{2,OH} = 2.4$ Hz, OH); 3.54 (m, 1H, H-2); 3.55 (s, 3H, OCH_3); 4.03 (m, 1H, $J_{5,6} = 6.2$ Hz, H-5); 4.09 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1); 4.12 (m, 2H, H-3, and H-4); 4.38 (m, 2H, H-6, and H-6′). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.25%; H, 7.95%. Found C, 55.19%; H, 8.09%.

Methyl 6-*O*-(2-methylpropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (5b). White solid, 95% yield, R_f 0.20 (4:6 hexane–EtOAc); mp 104–107 °C (from petroleum ether); $[\alpha]_D$ +21.7 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.19 (d, 6H, $J = 6.9$ Hz, $(CH_3)_2CH$); 1.34, and 1.52 (2 s, each 3H, $C(CH_3)_2$), 2.64 (e, 1H, *CH*CO); 2.75 (bs, 1H, OH); 3.54 (s, 3H, OCH_3); 3.54 (dd, 1H, $J_{2,3} = 6.9$ Hz, H-2); 4.01 (ddd, 1H, $J_{4,5} = 2.0$ Hz, $J_{5,6} = 6.3$ Hz, H-5); 4.09 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1); 4.11 (m, 2H, H-3, and H-4); 4.37 (m, 2H, H-6 and H-6′). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.25%; H, 7.95%. Found C, 55.32%; H, 8.05%.

Methyl 6-O-(2,2-dimethylpropanoyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (5c). White solid, 96% yield, R_f 0.29 (4:6 hexane–EtOAc); mp 125–129 °C (from petroleum ether); $[\alpha]_D$ +21.4 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.22 (s, 9H, $(CH_3)_3C$); 1.34, and 1.52 (2 s, each 3H, $C(CH_3)_2$); 2.65 (bs, 1H, OH); 3.55 (s, 3H, OCH_3); 3.55 (m, 1H, H-2); 3.97 (m, 1H, $J_{4,5} = 2.0$ Hz, $J_{5,6} = 6.4$ Hz, H-5); 4.09 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1); 4.10 (m, 2H, H-3, and H-4); 4.36 (d, 2H, H-6 and H-6′). Anal. calcd for $C_{15}H_{26}O_7$: C, 56.59%; H, 8.23%. Found C, 56.69%; H, 8.28%.

1-*O*-Acyl-2,3:5,6-di-*O*-isopropylidene-α- and β-D-mannofuranose (7a–c). To a stirred solution of dry 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**6**) (1.30 g, 1.0 mmol) in anhydrous toluene (15 mL), anhydrous Et_3N (1.75 mL, 12 mmol) and the required acyl chloride (11.0 mmol) were added. The reaction mixture was stirred under reflux until TLC analysis (1:1 hexane–EtOAc) revealed the complete disappearance of the starting material, filtered, and concentrated under reduced pressure. A flash chromatography on silica gel of these crude reaction products gave the desired 1-*O*-acyl derivatives **7**.

1-*O*-Butanoyl-2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (α-7a). Syrup, 95% yield, R_f 0.60 (1:1 hexane–EtOAc); $[\alpha]_D$ +40.8 (*c* 1.2, $CHCl_3$), lit:[24a] $[\alpha]_D$ +43.5 (*c* 1.1, $CHCl_3$).

1-*O*-(2-Methylpropanoyl)-2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (α-7b). Syrup, 72% yield, R_f 0.46 (1:1 hexane–EtOAc); $[\alpha]_D$ +40.8 (*c* 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.17 (d, 6H, $J = 6.9$ Hz, $(CH_3)_2CH$); 1.34, 1.37, 1.45, and 1.48 (4 s, each 3H, $2\times C(CH_3)_2$), 2.54 (m, 1H, *CH*CO); 4.00 (dd, 1H, $J_{6,6'} = 8.9$ Hz, $J_{5,6'} = 4.5$ Hz, H-6′); 4.00 (dd, 1H, $J_{4,5} = 7.9$ Hz, H-4); 4.09 (dd, 1H, $J_{5,6} = 6.1$ Hz, H-6); 4.39 (ddd, 1H, H-5); 4.70 (d, 1H, $J_{2,3} = 5.9$ Hz, H-2); 4.87 (dd, 1H, $J_{3,4} = 3.7$ Hz, H-3); 6.12 (s, 1H, H-1). Anal. calcd for $C_{16}H_{26}O_7$: C, 58.17%; H, 7.93%. Found C, 58.32%; H, 8.02%.

1-*O*-(2-Methylpropanoyl)-2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranose (β-7b). Syrup, 24% yield, R_f 0.37 (1:1 hexane–EtOAc); $[\alpha]_D$ +27.2 (*c* 1.0, $CHCl_3$). ^1H NMR (C_6D_6), δ: 1.03 (d, 3H, $J = 5.4$ Hz, CH_3CH); 1.07 (d, 3H, $J = 5.4$ Hz, CH_3CH); 1.13, 1.27, 1.42, and 1.45 (4 s, each 3H, $2\times C(CH_3)_2$), 2.40 (m, 1H, *CH*CO); 3.45 (dd, 1H, $J_{4,5} = 7.4$ Hz, H-4); 4.02 (dd, 1H, $J_{6,6'} = 8.7$ Hz, $J_{5,6'} = 6.4$ Hz, H-6′); 4.12 (dd, 1H, $J_{5,6} = 5.3$ Hz, H-6); 4.26 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3); 4.31 (dd, 1H, $J_{2,3} = 6.0$ Hz, H-2); 4.53 (ddd, 1H, H-5); 5.78 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1). Anal. calcd for $C_{16}H_{26}O_7$: C, 58.17%; H, 7.93%. Found C, 58.02%; H, 7.88%.

1-*O*-(2,2-Dimethylpropanoyl)-2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (α-7c). White solid, 37% yield, R_f 0.61 (1:1 hexane–EtOAc); mp 69–71 °C (directly from flash chromatography); $[\alpha]_D$ +49.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.20 (s, 9H, $(CH_3)_3C$); 1.35, 1.38, 1.46, and 1.50 (4 s, each 3H, $2\times C(CH_3)_2$); 3.97 (dd, 1H, $J_{4,5} = 8.0$ Hz, H-4); 4.00 (dd, 1H, $J_{6,6'} = 8.8$ Hz, $J_{5,6'} = 4.4$ Hz, H-6′); 4.11 (dd, 1H, $J_{5,6} = 6.1$ Hz, H-6); 4.40 (ddd, 1H, H-5); 4.69 (d, 1H, $J_{2,3} = 5.9$ Hz, H-2); 4.87 (dd, 1H, $J_{3,4} = 3.6$ Hz, H-3); 6.12 (s, 1H, H-1). Anal. calcd for $C_{17}H_{28}O_7$: C, 59.28%; H, 8.19%. Found C, 59.26%; H, 8.31%.

1-*O*-(2,2-Dimethylpropanoyl)-2,3:5,6-di-*O*-isopropylidene-β-D-mannofuranose (β-7c). White solid, 52% yield, R_f 0.51 (1:1 hexane–EtOAc); mp 67–68 °C (directly by flash-chromatography); $[\alpha]_D$ +25.0 (*c* 1.4, $CHCl_3$). ^1H NMR (C_6D_6), δ: 1.14, 1.26, 1.42, and 1.45 (4 s, each 3H, $2\times C(CH_3)_2$); 1.19 (s, 9H, $(CH_3)_3C$); 3.35 (dd, 1H, $J_{4,5} = 7.6$ Hz, H-4); 4.00 (dd, 1H, $J_{6,6'} = 8.6$ Hz, $J_{5,6'} = 6.3$ Hz, H-6′); 4.09 (dd, 1H, $J_{5,6} = 5.3$ Hz, H-6); 4.27 (dd, 1H, $J_{3,4} = 3.3$ Hz, H-3); 4.33 (dd, 1H, $J_{2,3} = 6.0$ Hz, H-2); 4.53 (ddd, 1H, H-5); 5.68 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1). Anal. calcd for $C_{17}H_{28}O_7$: C, 59.28%; H, 8.19%. Found C, 59.33%; H, 8.26%.

1-*O*-Acyl-2,3-*O*-isopropylidene-α- and β-D-mannofuranoses (8–9). To a solution of di-*O*-isopropylidene derivatives **7** (1.0 mmol) in EtOH (9 mL), copper(II) chloride dihydrate (850 mg, 4.9 mmol) was added at room temperature, and the reaction mixture was stirred at room temperature until TLC analysis (3:7 hexane–EtOAc) revealed the complete disappearance of the starting material (about 4 h for β anomers and about 16 h for α anomers). The reaction was stopped by addition

of solid $NaHCO_3$ (900 mg) and the mixture was stirred for 30 min until no more CO_2 was formed. Then, 1.0 mL of H_2O was added, a further amount of CO_2 being produced, and the mixture was diluted with 4.0 mL of H_2O to promote precipitation. The pale blue precipitate was filtered on Celite and the filtrate was extracted with $CHCl_3$ (3×10 mL). The organic extracts were combined, dried, concentrated under reduced pressure, and the resulting residue was directly subjected to flash chromatography on silica gel.

1-*O*-Butanoyl-2,3-*O*-isopropylidene-α-D-mannofuranose (8a). White solid, 80% yield, mp 76–77 °C (directly by flash chromatography) R_f 0.18 (95:5 CH_2Cl_2–MeOH); $[\alpha]_D$ +57.3 (*c* 1.5, $CHCl_3$); lit[24a]: $[\alpha]_D$ +54.6 (*c* 1.2, $CHCl_3$), mp 79 °C.

1-*O*-(2-Methylpropanoyl)-2,3-*O*-isopropylidene-α-D-mannofuranose (8b). White solid, 80% yield, R_f 0.45 (EtOAc); mp 98–99 °C (directly by flash-chromatography); $[\alpha]_D$ +58.3 (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.16 (d, 6H, J = 7.0 Hz, $(CH_3)_2CH$); 1.36, and 1.50 (2 s, each 3H, $C(CH_3)_2$), 2.05 (bs, 1H, OH); 2.52 (m, 1H, *CH*CO); 2.73 (bs, 1H, OH); 3.71 (dd, 1H, $J_{6,6'}$ = 11.42 Hz, $J_{5,6'}$ = 5.0 Hz, H-6′); 3.87 (dd, 1H, $J_{5,6}$ = 2.9 Hz, H-6); 4.04 (ddd, 1H, H-5); 4.05 (dd, 1H, $J_{4,5}$ = 8.1 Hz, H-4); 4.70 (d, 1H, $J_{2,3}$ = 5.9 Hz, H-2); 4.93 (dd, 1H, $J_{3,4}$ = 3.5 Hz, H-3); 6.17 (s, 1H, H-1). Anal. calcd for $C_{13}H_{22}O_7$: C, 53.78%; H, 7.64%. Found C, 53.86%; H, 7.76%.

1-*O*-(2,2-Dimethylpropanoyl)-2,3-*O*-isopropylidene-α-D-mannofuranose (8c). White solid, 80% yield, R_f 0.45 (EtOAc); mp 149 °C (directly by flash-chromatography); $[\alpha]_D$ +60.4 (*c* 1.1, $CHCl_3$). ^1H NMR (CD_3CN–D_2O), δ: 1.15 (s, 9H, $(CH_3)_3C$); 1.31, and 1.42 (2 s, each 3H, $C(CH_3)_2$); 3.45 (dd, 1H, $J_{6,6'}$ = 11.5 Hz, $J_{5,6'}$ = 5.6 Hz, H-6′); 3.63 (dd, 1H, $J_{5,6}$ = 2.9 Hz, H-6); 3.81 (ddd, 1H, H-5); 3.96 (dd, 1H, $J_{4,5}$ = 8.7 Hz, H-4); 4.67 (d, 1H, $J_{2,3}$ = 5.9 Hz, H-2); 4.87 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3); 5.94 (s, 1H, H-1). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.26%; H, 7.95%. Found C, 55.07%; H, 8.03%.

1-*O*-(2-Methylpropanoyl)-2,3-*O*-isopropylidene-β-D-mannofuranose (9b). Syrup, 50% yield, R_f 0.20 (EtOAc); $[\alpha]_D$ +11.4 (*c* 2.5, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.21 (d, 6H, J = 7.0 Hz, $(CH_3)_2CH$); 1.39, and 1.56 (2 s, each 3H, $C(CH_3)_2$), 2.10 (bs, 2H, OH); 2.62 (m, 1H, *CH*CO); 3.73 (dd, 1H, $J_{6,6'}$ = 11.5 Hz, $J_{5,6'}$ = 5.3 Hz, H-6′); 3.87 (dd, 1H, $J_{5,6}$ = 3.2 Hz, H-6); 3.97 (dd, 1H, $J_{4,5}$ = 8.9 Hz, H-4); 4.10 (ddd, 1H, H-5); 4.85 (dd, 1H, $J_{2,3}$ = 6.6 Hz, H-2); 4.94 (dd, 1H, $J_{3,4}$ = 4.6 Hz, H-3); 5.93 (d, 1H, $J_{1,2}$ = 4.2 Hz, H-1). Anal. calcd for $C_{13}H_{22}O_7$: C, 53.78%; H, 7.64%. Found C, 53.77%; H, 7.64%.

1-*O*-(2,2-Dimethylpropanoyl)-2,3-*O*-isopropylidene-β-D-mannofuranose (9c). Syrup, 40% yield, R_f 0.35 (EtOAc); $[\alpha]_D$ +16.4 (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 1.25 (s, 9H, $(CH_3)_3C$); 1.39, and 1.56 (2 s, each 3H, $C(CH_3)_2$); 1.99 (bs, 1H, OH); 2.90 (bs, 1H, OH); 3.73 (dd, 1H, $J_{6,6'}$ = 11.5 Hz, $J_{5,6'}$ = 5.3 Hz, H-6′); 3.88 (dd, 1H, $J_{5,6}$ = 3.1 Hz, H-6); 3.95 (dd, 1H, $J_{4,5}$ = 8.8 Hz, H-4); 4.08 (ddd, 1H, H-5); 4.86 (dd, 1H, $J_{2,3}$ = 6.9 Hz, H-2); 4.95 (dd, 1H, $J_{3,4}$ = 4.8 Hz, H-3); 5.90 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1). Anal. calcd for $C_{14}H_{24}O_7$: C, 55.26%; H, 7.95%. Found C, 55.35%; H, 7.89%.

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