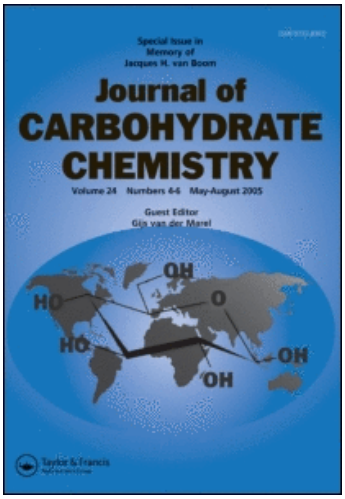

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The Synthesis of Allolactose from Amygdalin

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ABSTRACT

An original synthetic approach to the disaccharide allolactose **(1)** starting from the natural glycoside amygdalin **(2)** has been developed. The disaccharidic gentiobiose unit present in **2** has been transformed into the allolactose moiety using a four step protocol based on the selective inversion of the C-4′ OH. The efficient removal of the natural benzylic aglycone (a mandelonitrile moiety) was accomplished by catalytic transfer hydrogenolysis. The behavior of allolactose with different enzymes is also described.

Key Words: Allolactose; Amygdalin; Oxidoreductases; Analytical standard.

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INTRODUCTION

Allolactose (β-D-galactopyranosyl-(1→6)-α,β-D-glucopyranose, **1**) is a reducing disaccharide playing an important biological role in bacterial metabolism. For instance, in *E. coli* allolactose regulates the activity of the so-called ''lac-operon'',[1,2] which consists of three structural genes responsible for the uptake and hydrolysis of lactose used as a carbon source. In the absence of lactose a repressor protein is bound to the operator region of the lac operon, thus preventing RNA polymerase from binding to the DNA and initiating the transcription. In the presence of lactose, a β-galactosidase converts some lactose to allolactose **(1)**, which binds to the tetramer repressor and induces an allosteric change of this protein. As a consequence, the repressor protein is no longer able to bind to DNA, so it falls off exposing the operator and its promoter sequence for transcription.

Despite its biological importance and its simple structure, allolactose is presently not commercially available. Synthetic approaches to this molecule exploited either the usual protocols of chemical condensation between suitable galactose donors and glucose acceptors by Koenigs-Knorr glycosylation,[2] or – mimicking nature – a β-galactosidase catalyzed trans-glycosylation.[3,4] The latter approach led to the formation of a mixture of mono-, di- and trisaccharides that had to be carefully separated.

In the framework of an EEC-project pursuing enzymatic lactose valorization, we became interested in producing allolactose to be used as a chromatographic standard and as a potential substrate for newly isolated sugar-oxidoreductases. The use of natural building blocks can have some advantages for the synthesis of di- and oligosaccharides,[5,6] as already well-documented in the literature. Accordingly, we took into consideration the elaboration of a natural disaccharide derivative as a starting material, in order to avoid chemical or enzymatic glycosylation. The synthetic protocol that has been developed as well as the performances of allolactose with different enzymes are described in this report.

RESULTS AND DISCUSSION

Amygdalin **(2)** is a commercially available natural cyanogenic glycoside which occurs in the seeds of *Rosaceae*, principally in bitter almonds.[7] Its chemical structure appeared quite suitable for our purposes in that the disaccharidic unit might be chemically elaborated from the actual gentiobiose (β-D-glucopyranosyl-(1→6)-D-glucose) moiety to the desired allolactose (β-D-galactopyranosyl-(1→6)-D-glucose) target by selective inversion of the C-4′ OH followed by the removal of the natural benzylic aglycone (a mandelonitrile moiety) by catalytic hydrogenolysis.

Scheme 1 shows the optimized protocol that has been developed. The new compound **4** was prepared from amygdalin by a two-step standard procedure and its 4,6-*O*-benzylidene acetal was efficiently and selectively reduced following the protocol suggested by DeNinno et al. (triethylsilane – trifluoroacetic acid – trifluoroacetic anhydride)[8] to give the previously undescribed monohydroxy derivative **5** (the three synthetic steps gave **5** in 59% overall isolated yields from **2**) After different attempts, the inversion of the equatorial 4′-OH to the desired axial hydroxyl was accomplished *via* the triflate intermediate **5a** (not isolated), exploiting an approach already used for

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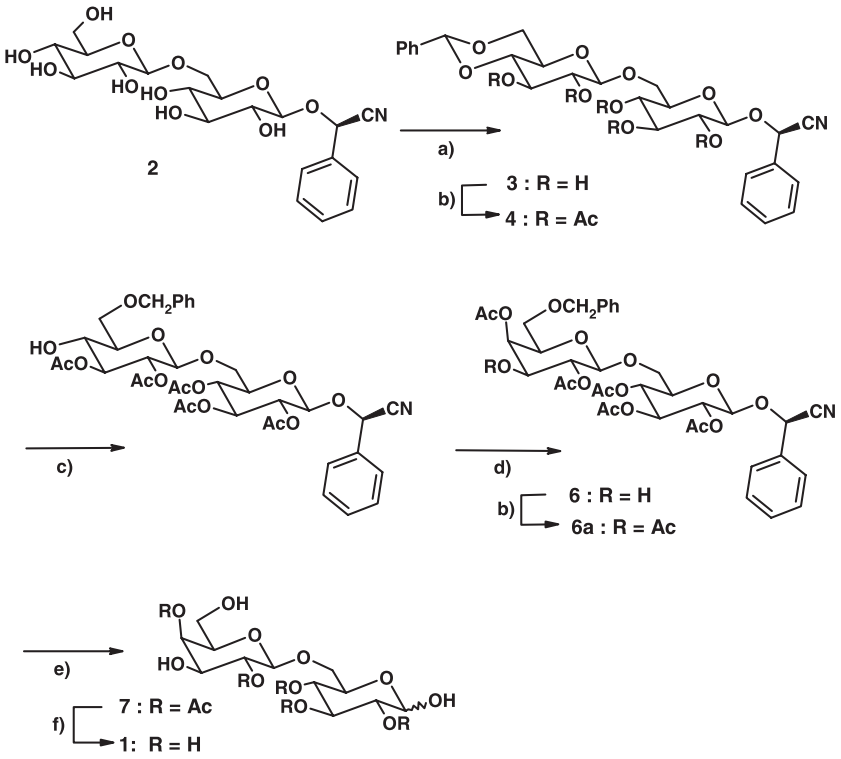


Scheme 1. Synthesis of allolactose from amygdalin. a) $PhCH(OMe)_2$, H^+, 35°C; b) Ac_2O, DMAP, Et_3N; c) Et_3SiH, Trifluroacetic anhydride, TFA; d) Tf_2O, −15°C; then H_2O, reflux; e) Cyclohexadiene, Pd/C, reflux; f) IRA-400 resin (OH^- form).

the inversion of C-4 OH of glucosamine.[9] This compound was prepared by reacting **5** with triflic anhydride in CH_2Cl_2; water was added to the reaction mixture which was then boiled to give **6** (inversion of the 4'-OH was accompanied by the migration of the 3′-OAc) as the main product in 67% isolated yields (**5** was also recovered in 22% yield, due to the undesired triflate hydrolysis).

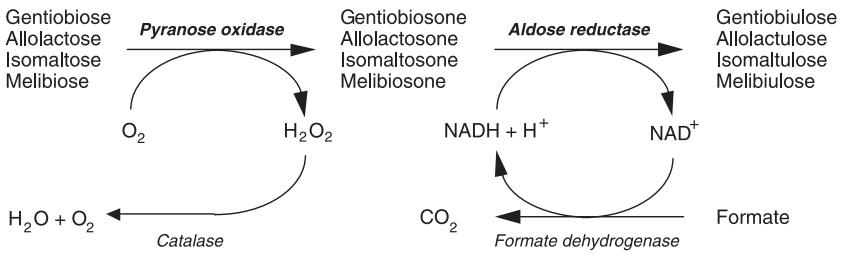
The presence of the axial acetate at C-4′ and of the non-acetylated OH at C-3′ was confirmed by NMR analysis. The ^1H NMR spectrum of the precursor gentiobiosyl derivative **5** showed five triplets between 4.85 and 5.11 ppm, due to H-3′, H-2′, H-4, H-3 and H-2. Only four triplets were present in the same region of the ^1H NMR spectrum of **6** and, in addition, a newly appeared broad doublet (J=3.9 Hz), integrated as one proton, resonated at 5.39 ppm, clearly due to the equatorial H-4′. A 2D-COSY experiment showed that this signal was coupled to a broad triplet at 3.81 ppm and to a doublet of doublet (J=8.6 Hz and J=3.9 Hz) at 3.90 ppm. The latter signal, additionally coupled to a triplet at 5.0 ppm (H-2′), was significantly (more than 1 ppm) downfield shifted upon acetylation (compound **6a**) and it was therefore assigned to the axial H-3′, while the broad triplet at 3.81 ppm was the signal due to H-5′.

The final deprotection of compound **6** was best achieved by catalytic transfer hydrogenation (substrate boiled in EtOH in the presence of 1,4-hexadiene and Pd/C)[10]

to remove the benzylic substituents (the mandelonitrile aglycone and the C-6′ benzyl group) to give the intermediate **7**, followed by methanolysis of the acetate groups in the presence of an anion exchange resin (OH^-). Both the reactions were quantitative and pure **1** was recovered by solvent evaporation.

The structure of **1** was proved to be allolactose by ^{13}C NMR. The presence of a reducing disaccharide was confirmed by the three anomeric signals at 106.2 (C-1′), 98.9 (C-1β) and 95.0 (C-1α) ppm. Additionally, a diagnostic upfield-shifted signal at 71.5 ppm, due to the C-4′ of a galactose moiety, was observed. All these data were in full agreement with a literature report.[11]

Allolactose was then tested for different applications. Initially, it was a useful standard for the analysis and the identification (by capillary electrophoresis) of a mixture of oligosaccharides produced by transglycosylation of lactose with thermostable β-glycosidases.[12] Secondly, **1** was used for investigating the substrate specificity of disaccharide-converting enzymes. The first biocatalyst considered was a cellobiose dehydrogenase, an enzyme that is produced extracellularly by a number of wood- and cellulose-degrading fungi when grown on cellulose, and that, in our case, was isolated from the plant pathogen *Sclerotium* (*Athelia*) *rolfsii*.[13] These biocatalysts oxidize the reducing end of cellobiose and cello-oligosacharides to their corresponding 1,5-lactones, which are subsequently hydrolyzed to the corresponding carboxylic acids in aqueous environments. Knowledge of the specificity of these enzymes is important since they might be useful for the specific determination of lactose, e.g., in samples of oligosaccharides produced with the above described process,[12] in view of lactose intolerance of consumers that might use these oligosaccharide mixtures. It was found that the cellobiose dehydrogenase from *Sclerotium* (*Athelia*) *rolfsii* is indeed very specific for reducing disaccharides carrying a β-1,4-glycosidic linkage: cellobiose and lactose were converted to the corresponding bionic acid, while neither allolactose nor other β-1,6- (gentiobiose), β-1,2- (sophorose) and β-1,3 positional isomers of cellobiose and lactose were oxidized.[13] Based on these results the application of cellobiose dehydrogenase for the analytic purpose outlined should be feasible. Finally, allolactose was regioselectively oxidized at C-2 OH by a pyranose 2-oxidase isolated from the basidiomycete *Trametes multicolor*.[14] This enzyme catalyzes the oxidation of several aldopyranoses at the C-2 position to yield the corresponding aldos-2-uloses (osones). As shown in Scheme 2, this was the first step of an enzymatic isomerization of a series of aldose-disaccharides into the corresponding ketoses, a transformation that cannot be



Scheme 2. Isomerization of aldose-disaccharides into the corresponding ketones by an oxidoreductive route employing pyranose oxidase and aldose reductase (see Ref. [14]).

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catalyzed by the available glucose (xylose) isomerases, enzymes that are used for the conversion of D-glucose into D-fructose. Four 1,6-linked disaccharides (allolactose and the commercially available gentiobiose, isomaltose [α-D-Glc*p*-(1→6)-D-Glc] and melibiose [α-D-Gal*p*-(1→6)-D-Glc]), were converted into the corresponding glycosyl D-*arabino*-hexos-2-uloses. Gentiobiose and allolactose were clearly the preferred substrates showing, after 2 hours, a 90 and 72% conversion, respectively, while melibiose and isomaltose required much longer reaction times.[14]

In conclusion, we have reported an original synthetic approach to the disaccharide allolactose (which avoids the use of heavy metal salts, as in the Koenigs-Knorr glycosylation, or tedious chromatographic separation, as in the glycosidases-catalyzed transglycosylation) and we have summarized the performances of different enzymes with this disaccharide.[13,14] The facile removal of the mandelonitrile aglycone (that might be considered as a ''natural'' anomeric protective group) and the easy access to partly protected intermediates, such as **5** and **6**, which might be useful for the synthesis of more complex derivatives, make amygdalin an interesting and available building block for oligosaccharides carrying a reducing gentiobiose or allolactose moiety.

EXPERIMENTAL

General methods. Amygdalin **(2)** and the other reagents were obtained from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300 instrument. $[\alpha]_D$ were measured using a Perkin-Elmer 141 polarimeter. Melting points were determined using a Kofler apparatus. TLC was performed on Merck Silica Gel 60 F_{254} plates.

4′,6′-*O*-Benzylidene amygdalin (3). Amygdalin **2** (4 g, 9 mmol) was dissolved in dry dimethylformamide (20 mL). Benzaldehyde dimethyl acetal (3.3 mL, 22 mmol) and a catalytic amount of *p*-toluenesulfonic acid hydrate (50 mg) were added to the solution in a round-bottomed flask, which was then attached to a rotary rotavapor immersed in a water bath at 35°C. The reaction was completed in 4 hours (TLC: AcOEt/MeOH/H_2O, 80:20:5 v/v). Et_3N (1 mL) was added and the solvent was then evaporated at reduced pressure. The solid obtained after evaporation was dissolved in hot methanol (10 mL) (if necessary, some drops of water could be added) and allowed to precipitate overnight, giving **3** as a white solid. Yield 4.35 g (91%); mp 232–234°C; $[\alpha]_D^{20} = -68.0°$ (*c* 0.50, DMSO); ^{1}H NMR (D_6-DMSO+D_2O): δ 7.7–7.2 (m, 10 H, Ar), 5.98 (s, 1 H, CHCN), 5.61 (s, 1 H, PhCH), 4.66 and 4.38 (2 d, 1 H each, *J* 8.5 Hz and *J* 8.0, H-1 and H-1′), 4.25 (dd, 1 H, *J* 10.5 Hz, *J* 4.0 Hz, H-6′$_{eq}$), 4.09 (d, 1 H, *J* 11.5 Hz, H-6a), 3.8–3.1 (several m, 10 H).

Anal. Calcd for $C_{27}H_{31}NO_{11}$: C, 59.45; H, 5.69; N, 2.57. Found: C, 59.57; H, 5.64; N, 2.62.

2,3,4,2′,3′-Penta-*O*-acetyl-4′,6′-*O*-benzylidene amygdalin (4). Compound **3** (4.25 g, 8 mmol) was dissolved in 100 mL of CH_2Cl_2. Dimethylaminopyridine (150 mg) and Et_3N (11 mL, 79 mmol) were added and the mixture was cooled at 0°C. Acetic anhydride (15 mL, 158 mmol) was added dropwise at 0°C. When the addition

was terminated, the mixture was allowed to react for 2 h at room temperature (TLC: AcOEt/hexane 4:6). The mixture was washed with H_2O (80 mL), 5% w/v $NaHCO_3$ (80 mL) and H_2O again (80 mL), until the pH was neutral. The organic solution was separated from the aqueous phase, dried with Na_2SO_4 and concentrated at reduced pressure, providing the product **4** as a white solid (5.2 g, 88% yield): mp 183–184°C; $[\alpha]_D^{20} = -66.2°$ (*c* 0.50, $CHCl_3$,); ^{1}H NMR ($CDCl_3$): δ 7.5–7.3 (m, 10 H, ArH), 5.56 and 5.49 (2 s, 1 H each, CHCN and PhCH), 5.33 (t, 1 H, *J* 9,8 Hz) and 5.14–4.89 (m, 4 H) : H-2, H-3, H-4, H-2′, H-3′, 4.69 and 4.46 (2 d, 1 H each, *J* 7.9 Hz, H-1 and H-1′), 4.37 (dd, 1 H, *J* 10.0 Hz, *J* 4.4 Hz, H-6′$_{eq}$), 3.92–3.49 (several m, 6 H), 2.10, 2.04, 1.99, 1.97 (4 s, 15 H total, Ac).

Anal. Calcd for $C_{37}H_{41}NO_{16}$: C, 58.81; H, 5.43; N, 1.85. Found: C, 58.71; H, 5.54; N, 1.88.

2,3,4,2′,3′-Penta-*O*-acetyl-6′-*O*-benzyl amygdalin (5). Carefully dried compound **4** (5.2 g, 7 mmol) was dissolved in dry CH_2Cl_2 (30 mL) in an inert atmosphere at 0°C. Triethylsilane (5.7 mL, 36 mmol), trifluoroacetic anhydride (3.0 mL, 21 mmol) and trifluoroacetic acid (2.7 mL, 36 mmol) were added dropwise. After stirring at room temperature for 1 h (TLC: AcOEt/hexane 1:1), the solution was diluted with CH_2Cl_2 (100 mL) and washed successively with H_2O (60 mL), 5% $NaHCO_3$ (60 mL) and H_2O again (20 mL). The organic layer was dried with Na_2SO_4 and the solvent was evaporated. The product was purified by flash chromatography (AcOEt/hexane 1:1), furnishing **5** as a white solid in 74% yield (3.85 g): mp168–169°C; $[\alpha]_D^{20} = -47.1°$ (*c* 0.50, $CHCl_3$,). ^{1}H NMR ($CDCl_3$): δ 7.5–7.2 (m, 10 H, ArH), 5.53 (s, 1H, CHCN), 5.12–4.85 (m, 5 H, H-2, H-3, H-4, H-2′, H-3′), 4.61 and 4.54 (2 d, 1 H each, *J* 12.2 Hz, $PhCH_2$), 4.58 and 4.39 (2 d, 1 H each, *J* 8.0 Hz, H-1 and H-1′), 3.89–3.51 (several m, 8 H), 2.10, 2.09, 1.99, 1.98 (4 s, 15 H total, Ac); ^{13}C NMR ($CDCl_3$): δ 171.3 (CO), 170.1 (CO), 169.8 (CO), 169.4 (CO), 168.9 (s, CO), 137.7, 132.1, 130.4, 129.2, 128.4, 127.7, 117.1 (CN), 100.5 and 97.6 (C-1 and C1'), 75.4, 74.2, 73.7 (Ph*C*H_2), 73.6, 72.5, 71.3, 70.9, 70.4, 69.9, 68.7, 67.8, 67.6, 20.8, 20.4.

Anal. Calcd for $C_{37}H_{43}NO_{16}$: C, 58.65; H, 5.68; N, 1.85. Found: C, 58.64; H, 5.74; N, 1.86.

Phenyl cyanomethyl 2′,4′-di-*O*-acetyl-6′-*O*-benzyl-β-D-galactopyranosyl-(1→6)-(2,3,4-tri-*O*-acetyl-β-D-glucopyranoside) (6). Compound **5** (300 mg, 0.40 mmol) was dissolved in dry CH_2Cl_2 (6 mL) under an inert atmosphere. Pyridine (200 μL, 2.48 mmol) was added and the mixture was cooled at −15°C. Triflic anhydride (180 μL, 1.07 mmol) was added dropwise and the formation of the triflate **5a** was observed in TLC (eluent AcOEt/hexane 1:1). When the reaction was complete (1 h), H_2O was added (250 μL) and the resulting solution was refluxed for 4 h. Subsequently, the mixture was diluted with CH_2Cl_2 (20 mL), washed with 5% HCl (10 mL), $NaHCO_3$ (20 mL) and H_2O (20 mL). The organic solution was dried with Na_2SO_4 and concentrated at reduced pressure. The product was purified by flash chromatography (AcOEt/hexane 1:1), furnishing **6** as a white solid in 67% yield (200 mg) together with 66 mg of starting compound **5**. Compound **6**: mp 165–166°C; $[\alpha]_D^{20} = -40.5°$ (*c* 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$, COSY): δ 7.5–7.2 (m, 10 H, ArH), 5.54 (s, 1 H, CHCN), 5.38 (br d, 1 H, *J* 3.8 Hz, H-4′), 5.12–4.97 (m, 3 H, H-2, H-3, H-2′), 4.91 (br t, 1 H, *J* 8.8 Hz, H-4),

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4.55 and 4.41 (2 d, 1 H each, *J* 8.0 Hz, H-1 and H-1′), 4.53 and 4.44 (2 d, 1 H each, *J* 11.6 Hz, $PhCH_2$), 3.95–3.85 (m, 2 H, H-3′ and H-6a), 3.81 (br t, 1H, *J* 6.5 Hz, H-5′), 3.68–3.50 (m, 4H, H-5, H-6b, H-6′a, H-6′b), 2.15, 2.09, 1.96, 1.95, 1.93 (5 s, 15 H total, Ac). ^{13}C NMR ($CDCl_3$): δ 171.1 (CO), 171.0 (CO), 170.2 (CO), 169.5 (CO), 169.0 (CO), 132.2, 130.4, 129.3, 128.5, 127.9, 127.8, 117.1 (CN), 100.9 and 97.9 (C-1 and C1′), 73.7, 73.6, (Ph*C*H_2), 72.6, 72.5, 71.0, 70.2, 68.8, 68.0 (C-6 and C-6′), 67.8 (d), 21.1, 20.8, 20.5;

Anal. Calcd for $C_{37}H_{43}NO_{16}$: C, 58.65; H, 5.68; N, 1.85. Found: C, 58.68; H, 5.71; N, 1.85.

Conventional acetylation gave phenyl cyanomethyl 2′,3′,4′-di-*O*-acetyl-6′-*O*-benzyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (6a) as an oil. ^1H NMR ($CDCl_3$, COSY) : δ 7.5–7.2 (m, 10 H, ArH), 5.59 (s, 1 H, CHCN), 5.48 (br d, 1 H, *J* 3.6 Hz, H-4'), 5.21 (dd, 1 H, *J* 9.4 Hz, *J* 7.8 Hz, H-2′), 5.11–4.96 (m, 3 H, H-2, H-3, H-3′), 4.90 (br t, 1 H, *J* 8.8 Hz, H-4), 4.55 (d,1 H, *J* 7.8 Hz, H-1′), 4.53 and 4.41 (2 d, 1 H each, *J* 11.9 Hz, $PhCH_2$), 4.36 (d, 1H, *J* 8.0 Hz, H-1), 3.95–3.84 (m, 2 H, H-5′ and H-6a), 3.68–3.45 (m, 4H, H-5, H-6b, H-6′a, H-6′b), 2.11, 2.07, 1.98, 1.95 (4 s, 18 H total, Ac).

Anal. Calcd for $C_{39}H_{45}NO_{17}$: C, 58.57; H, 5.63; N, 1.75. Found: C, 58.65; H, 5.67; N, 1.73.

Allolactose (β-D-galactopyranosyl-(1→6)-α,β-D-glucopyranose, 1). Compound **6** (50 mg, 0.066 mmol) was dissolved in absolute ethanol by refluxing under nitrogen atmosphere. Pd/C (50 mg) and cyclohexadiene (300 μL, 3.171 mmol) were added and the solution was heated at 90°C. After 30 min the phenyl cyanomethyl aglycone was removed first (after approximately 30 min) and 8 h later the benzyl group was also completely eliminated (TLC: AcOEt). The product was filtered over packed celite and dried. The resulting solid intermediate compound **7** (37 mg, 0.066 mmol) was dissolved in methanol (1 mL) and complete deacetylation was accomplished in 1 h by adding an Amberlite IRA-400 resin (OH^- form) (TLC: AcOEt/MeOH/H_2O 8:3:1). The resin was filtered and the solvent evaporated. The reaction proceeded quantitatively providing 21 mg (0.061 mmol) of **1** as a pure white solid: mp 174–176°C (lit.[15] 174–176°C); $[\alpha]_D^{20} = 52.1°$ (*c* 0.33, H_2O) 5 min after soln (lit.[15] 54.2°), 32.0° after 1h, 26.3 after 24 h (lit.[15] 30.7°); ^{13}C NMR (D_2O): δ 106.2 (C-1′), 98.9 (C-1β), 95.0 (C-1α), 78.6 (C-3β), 78.0 (C-5), 77.8 (C-5β), 76.9 (C-2β), 75.6 (C-3′ and C-3α), 74.3 (C-2α), 73.6 (C-2′), 73.4 (C-5α), 72.4 (C-4α and C-4β), 71.5 (C-4′), 71.6 and 71.4 (C-6α and C-6β), 64.0 (C-6′); (Lit.:[11] 100 MHz, D_2O: δ 106.1 (C-1′), 98.8 (C-1β), 94.9 (C-1α), 78.5 (C-3β), 78.0 (C-5′); 77.7 (C-5β), 76.9 (C-2β), 75.5 (C-3′ and C-3α), 74.2 (C-2α), 73.6 (C-2′), 73.3 (C-5α), 72.3 (C-4α and C-4β), 71.5 (C-4′, C-6α and C-6β), 63.8 (C-6′).

Anal. Calcd for $C_{12}H_{22}O_{11}$: C, 42.11; H, 6.43. Found: C, 42.12; H, 6.48.

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REFERENCES

1. Jacob, F.; Monod, J. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **1961**, *3*, 318–356.
2. Jobe, A.; Bourgeois, S. *lac* Repressor-operator interaction VI. The natural inducer of the *lac* operon. J. Mol. Biol. **1972**, *69*, 397–408.
3. Huber, R.E.; Wallenfels, K.; Kurz, G. The action of β-galactosidase (*Escherichia coli*) on allolactose. Can. J. Biochem. **1975**, *53*, 1035–1038.
4. Pazur, J.H.; Tipton, C.L.; Budovich, T.; Marsh, J.M. Reversible transglycosylation. J. Am. Chem. Soc. **1958**, *80*, 119–121.
5. Rencurosi, A.; Poletti, L.; Panza, L.; Lay, L. Improvement on lipase catalysed regioselective *O*-acylation of lactose: a convenient route to 2′-*O*-fucosyllactose. J. Carbohydr. Chem. **2001**, *20*, 761–765.
6. La Ferla, B.; Lay, L.; Russo, G.; Panza, L. Regioselective lipase acylation as a useful tool for separation and selective protection of β-D-Gal(1→4)-D-GlcNAc and β-D-Gal(1→3)-D-GlcNAc disaccharides. Tetrahedron: Asymmetry **2000**, *18*, 3647–3651.
7. *The Merck Index*, 13th Ed.; Merck Research Laboratories, Division of Merck & Co., Inc.: Whitehouse Station NJ, USA, 2001; 100, n. 601.
8. De Ninno, M.P.; Etienne, J.B.; Duplantier, K.C. A method for the selective reduction of carbohydrate 4,6-*O*-benzylidene acetals. Tetrahedron Lett. **1995**, *36*, 669–672.
9. Lay, L.; Nicotra, F.; Panza, L.; Russo, G.; Adobati, E. Synthesis of the propyl glycoside of the trisaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc, component of a tumor antigen recognized by the antibody MBr1. Helv. Chim. Acta **1994**, *77*, 509–514.
10. Brieger, G.; Nestrick, T.J. Catalytic transfer hydrogenation. Chem. Rev. **1974**, *74*, 567–580.
11. Suyama, K.; Adachi, S.; Toba, T.; Sohma, T.; Hwang, C.-J.; Itoh, T. Isoraffinose (6^G-β-galactosylsucrose) synthesized by the intermolecular transglycosylation reaction of *Escherichia coli* β-galactosidase. Agric. Biol. Chem. **1986**, *50*, 2069–2075.
12. Petzelbauer, I.; Zeleny, R.; Reiter, A.; Kulbe, K.D.; Nidetzky, B. Development of an ultra-high-temperature process for the enzymatic hydrolysis of lactose: II. Oligosaccharide formation by two thermostable β-glycosidases. Biotechnol. Bioeng. **2000**, *69*, 140–149.
13. Baminger, U.; Subramaniam, S.S.; Renganathan, V.; Haltrich, D. Purification and characterization of cellobiose dehydrogenase from the plant pathogen *Sclerotium* (*Athelia*) *rolfsii*. Appl. Environ. Microbiol. **2001**, *67*, 1766–1774.
14. Leitner, C.; Mayr, P.; Riva, S.; Volc, J.; Kulbe, K.D.; Nidetzky, B.; Haltrich, D. Enzymatic redox isomerization of 1,6-disaccharides by pyranose oxidase and NADH-dependent aldose reductase. J. Mol. Catal., B Enzym. **2001**, *11*, 407–414.
15. Burckhardt, H.; Sparmberg, G. Crystallized 6-β-D-galactosido-D-glucose. Ber. **1933**, *66B*, 806–807.

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