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# Acetylesterase From Orange Peel as Biocatalyst for the Chemo- and Regioselective Deprotection of Carbohydrates

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**Abstract**—The enzyme acetylesterase from the *flavedo* of oranges (EC 3.1.1.6) can advantageously be applied for the chemo- and regioselective deprotection of different types of carbohydrates. It displays a selectivity which in many cases is complementary to the application of other biocatalysts or classical chemical methods.

## Introduction

In addition to the stereoselectivity which is characteristic for many enzyme-catalyzed reactions, the pronounced chemo- and regioselectivity displayed by numerous biocatalysts can also be exploited advantageously for the development of new synthetic methods which open up new and viable alternatives to established classical chemical transformations.[1] In particular, enzyme-catalyzed reactions have been applied for the selective introduction and/or removal of protecting functions under the mildest conditions in the chemistry of peptides, carbohydrates, alkaloids and steroids.[2] For instance, the selectivity of lipases and some proteases has been used in carbohydrate chemistry.[2] However, monosaccharides and, even more so, oligosaccharides and conjugates between carbohydrates and other classes of compounds, e.g. peptides, are multifunctional compounds which usually embody several (un)protected hydroxyl- and further functional groups. For their directed manipulation an arsenal of complementary synthetic techniques is required.[3] Therefore, the introduction of new biocatalysts with substrate tolerances differing from those of the enzymes mentioned above is of great interest to carbohydrate chemistry in particular and to protecting group chemistry in general.

In this paper we report that the enzyme acetylesterase from the flavedo of oranges (EC 3.1.1.6) is such a biocatalyst which can be employed advantageously for the chemo- and regioselective removal of acetyl groups from different types of carbohydrates.[4]

## Results and Discussion

The commercially available (Sigma) citrus acetylesterase from the peel of oranges (the major portion is found in the *flavedo*) was first described by E.F. Jansen *et al.*,[5] who had already found that the esterase preferably hydrolyzes esters of acetic acid, and that it accepts peracetylated polyols and selected carbohydrates as substrates. However, to the best of our knowledge, this biocatalyst has not been used before for preparative purposes. In order to determine its applicability for the removal of acetyl groups (the standard acyl protecting functions of carbohydrate chemistry)[3,6] from different pentoses and hexoses, several different monosaccharides were investigated as substrates for this biocatalyst.

### *Effect of cosolvents on the enzymatic activity*

Since peracylated carbohydrates, in general, are only sparingly soluble in aqueous solutions the effect of different solubilizing cosolvents on the activity of the enzyme was determined in order to find reaction conditions under which the possible substrates would be better accessible to acetylesterase. To this end, glyceroltriacetate (triacetin) was hydrolyzed enzymatically in mixtures of 0.15 N NaCl buffer and varying amounts of acetone, methanol, DMF, acetonitrile or dioxane at pH 6.5 and room temperature (Table 1). The observed initial rates of the hydrolyses demonstrate that the acetylesterase tolerates only relatively small amounts of organic cosolvents. Thus, in the presence of more than 10% (v/v) of acetone, acetonitrile or dioxane most of the enzymatic activity is lost. However, if 20% (v/v) of methanol or 15% (v/v) of DMF respectively, are added, 40–48 % of the activity is retained. Due to this unfavourable influence of the investigated cosolvents, the enzymatic hydrolyses on the preparative scale were carried out in 0.15 N NaCl buffer solution.

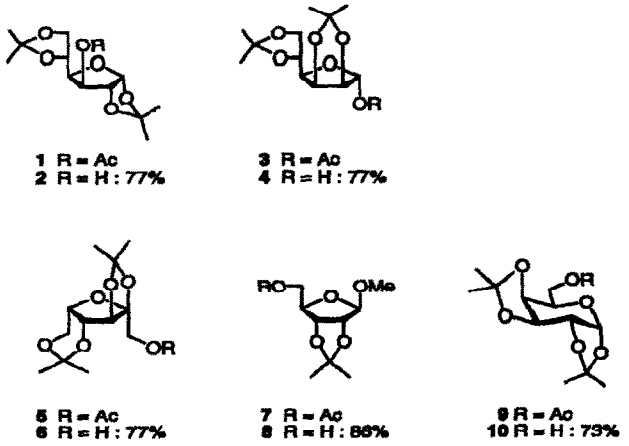
**Table 1.** Influence of organic cosolvents on the activity of acetyl esterase

| cosolvent | amount (vol %) | relative initial rates* [ %] |
|---|---|---|
| acetone | 10 | 90 |
| | 25 | 0 |
| | 50 | 0 |
| methanol | 10 | 75 |
| | 20 | 40 |
| | 25 | 8 |
| | 35 | 0 |
| DMF | 10 | 95 |
| | 15 | 48 |
| | 20 | 12 |
| acetonitrile | 10 | 10 |
| dioxane | 10 | 32 |

*Relative initial rate of the hydrolysis of the glycerol triacetate in 0.15 N NaCl buffer at pH 6.5 and rt (100 % = hydrolysis without any cosolvent).

*Chemoselective deacylation of isopropylidene-protected furanoses and pyranoses*

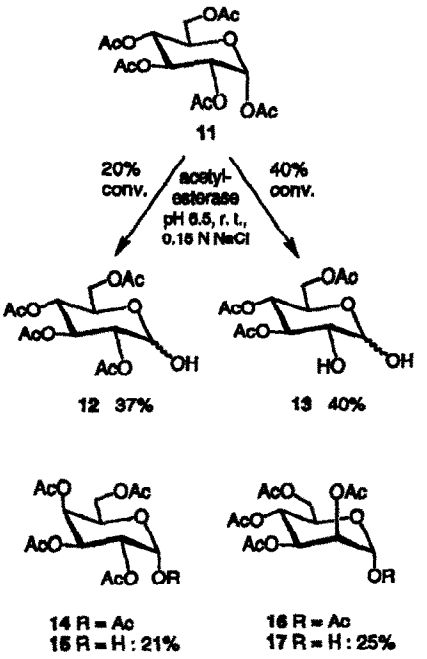
To investigate the chemoselective mode of action of acetylesterase, the furanoses **1**, **3**, **5**, and **7** and the hexose **9**, carrying acid-labile acetal protecting groups were subjected to the enzymatic hydrolysis. All compounds were accepted as substrates by the biocatalyst and the acetates were attacked with acceptable initial rates (Scheme I, Table 2, entries 1–5). In all cases a quantitative conversion was observed, the selectively deprotected carbohydrates **2**, **4**, **6**, **8** and **10** were isolated in yields of 73–86 % after flash chromatography. The conditions of the enzymatic transformations are so mild that the acid-labile isopropylidene acetals remain totally unaffected. Also, the enzyme displays a broad substrate tolerance. Thus, it not only attacks the primary acetates in **5**, **7** and **9**, but also the anomeric acetic acid ester in **3** and the sterically hindered acetate in diacetone glucose **1**.




Scheme I.

*Regioselective deacylation of peracetylated hexoses*

Besides chemoselectivity, the regioselective manipulation of blocking functions is of major relevance to protecting group chemistry.[3] To address this issue, pentaacetylglucose **11**, -galactose **14** and -mannose **16** were partially deprotected by means of acetylesterase. Again, all three compounds were hydrolyzed at acceptable rates (Scheme II, Table 2, entries 6–8). In each case the biocatalyst initially removes the anomeric acetic acid ester, so that at a conversion of 20 % (i.e. sufficient to remove only one acetate) the tetraacetates **12**, **15** and **17** are obtained as the major components of the resulting product mixtures. In the transformation of pentacetylglucose **11**, 17 % of the 3,4,6-triacetate **13** could also be isolated.




Scheme II.

In the cases of the galactose derivative **14** and the mannose derivative **16** a more complex product pattern was formed, from which no further hydrolysis product could be obtained in pure form.

**Table 2.** Relative initial rates of the chemo- and regioselective hydrolysis of different acetylated carbohydrates by acetylesterase

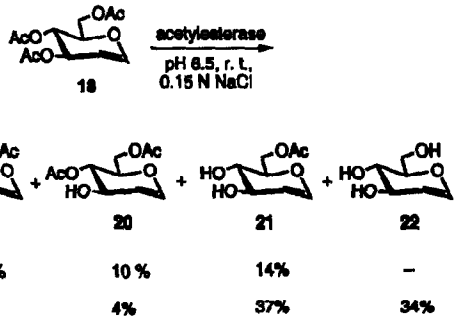
| Entry | substrate | number | relative initial rate* |
|---|---|---|---|
| 1 | 3-O-acetyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose | 1 | 5 % |
| 2 | 1-O-acetyl-2,3:5,6-di-O-isopropylidene-α-D-mannofuranose | 3 | 46 % |
| 3 | 1-O-acetyl-2,3:4,6-di-O-isopropylidene-β-L-sorbofuranose | 5 | 3 % |
| 4 | methyl-5-O-acetyl-2,3-O-isopropylidene-β-D-ribofuranosid | 7 | 7 % |
| 5 | 6-O-acetyl-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose | 9 | 3 % |
| 6 | 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose | 11 | 20 % |
| 7 | 1,2,3,4,6-penta-O-acetyl-α-D-galactopyranose | 14 | 9 % |
| 8 | 1,2,3,4,6-penta-O-acetyl-α-D-mannopyranose | 15 | 36 % |
| 9 | 3,4,6-tri-O-acetyl-D-glucal | 18 | 20 % |
| 10 | 1,6-anhydro-2,3,4-tri-O-acetyl-β-D-glucopyranose | 23 | 14 % |

*Relative initial rate of the hydrolysis at pH 6.5 in 0.15 N NaCl buffer (100 % = hydrolysis of glycerol triacetate under identical conditions).

If the deprotection of pentaacetylglucose **11** is allowed to proceed to 40 % conversion, the 2,3,4-triacetate **13** is formed as the major product in 40 % yield, and the tetraacetate **12** is still isolated with a yield of 12 %. The structure of **13** was determined by means of its $^1$H NMR COSY-spectrum and comparison with the spectra recorded for **11** and **12**. In **13**, the signals for 1-H and 2-H are shifted upfield as compared to the δ values for 1-H and 2-H in **11** (see Experimental). The regioselectivity displayed in this latter transformation stands in marked contrast to the reactivity of the acetyl protecting groups in classical chemical[6] as well as in different enzymatic[7] deprotections. Under the usual deblocking conditions employing bases, the acetates at the anomeric centre and at C-6 are the most reactive. We have, however, not determined whether the enzyme attacks the acetate at C-2 or whether the acetyl group migrates from O-2 to O-1 first[8] and is then cleaved off by the biocatalyst.

*Regioselective deacylation of tri-O-acetyl-D-glucal*

Glycals like **18** can advantageously be employed as versatile starting materials in 'ex-chiral-pool' syntheses of natural products[9] and in glycosylations.[10] Therefore, their regioselective manipulation is of current interest.[11] If tri-*O*-acetyl-D-glucal **18** is subjected to the action of acetylesterase from orange peel a relatively fast hydrolysis is observed (Scheme III, Table 2, entry 9). After 33 % conversion (i.e. sufficient to remove a single acetate group), the 3,6-diacetate **19** is isolated as the major product. In addition, the 4,6-diacetate **20** (10 %) and the 6-acetate **21** (14 %) are obtained in analytically pure form by chromatographic separation. If the conversion is extended to 66 %, the 6-acetate **21** is formed in 37 %, the diesters **19** and **20** are only present in small amounts. In addition, substantial amounts of the free glucal **22** are already formed. The structures of **19**–**21** were ascertained by means of their NMR spectra. Thus, the acetylesterase mediated hydrolysis makes the diacetate **19** which is selectively deprotected in the 4-position and the monoacetate **21** which carries only a blocking group on the primary 6-OH available in a straight-forward manner. The regioselectivity displayed by the esterase again contrasts favourably with the application of classical chemical manipulations and the use of different enzymes, e.g. lipases,[11] by which the primary 6-acetate is generally removed first.




Scheme III.

*Regioselective deacylation of tri-O-acetyl-1,6-anhydro-D-glucose*

1,6-Anhydro sugars are versatile intermediates which are employed for different purposes in carbohydrate chemistry,[12] e.g. in glycoside synthesis. Therefore, the regioselective removal of acetates from peracetylated 1,6-anhydro glucose **23** by means of citrus-acetylesterase was investigated.[13] This bicyclic compound, too, is accepted by the enzyme as a substrate (Scheme IV, Table 2, entry 10) and hydrolyzed at a reasonable rate.

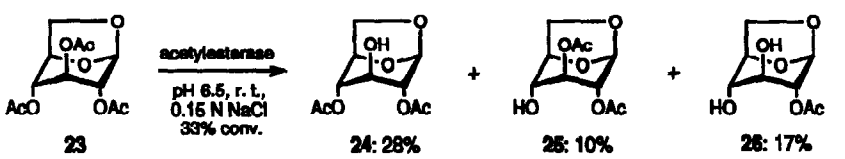
Surprisingly, the biocatalyst attacks the acetic acid ester at the sterically most congested 3-position predominantly. Thus, after 33 % conversion, the 2,4-diacetate **24** is isolated as the predominating product. Also, the axial protecting group at O-4 is removed preferably by acetylesterase, resulting in the formation of the 2,3-diester **25** in 10 % yield and the monoacetate **26** in 17 % yield.

This regioselectivity once more is different from the behaviour usually displayed by lipases, since these enzymes in the majority of the investigated cases attack the sterically better accessible blocking groups at the 2- and 4-substituents of the anhydropyranose.[13]

In conclusion, the orange peel acetylesterase reported in this paper proves to be an advantageous biocatalyst for the chemo- and regioselective removal of acetyl protecting groups from carbohydrates. It accepts a variety of differently functionalized pentoses and hexoses as well as unsaturated and bicyclic sugars. The enzyme splits off the acetic acid esters under mildest conditions, i.e. no undesired attack on acetals and enol ethers occurs, and displays a regioselectivity which is complementary to other established classical chemical and biocatalytic techniques.

## Experimental

$^1$H and $^{13}$C NMR spectra were recorded on Bruker spectrometers WH 90, AC 200, AC 250 or AM 400. Flash column chromatography was carried out on columns packed with Baker silica gel (30–60 μm). Thin layer chromatography on Kieselgel 60 $F_{254}$ aluminium sheets




Scheme IV.

(Merck, Darmstadt, Germany) was used to monitor the reactions and to ascertain the purity of the products. The enzyme acetylesterase from the *flavedo* of oranges was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). One unit of acetylesterase will produce 1.0 µm of acetic acid from glycerol triacetate per minute at pH 6.5 at 30°C. 3,4,6-Tri-*O*-acetylglucal was purchased from Fluka AG, Buchs, Switzerland.

The following compounds were prepared according to known procedures: 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**),[14] 1-*O*-acetyl-2,3:5,6-di-*O*-isopropylidene-α-D-manno-furanose (**3**),[14] 1-*O*-acetyl-2,3:4,6-di-*O*-isopropylidene-β-L-sorbofuranose (**5**),[14] methyl 5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (**7**),[15] 6-*O*-acetyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**9**),[14] 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**11**),[16] 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose (**14**),[16] 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose (**16**),[16] 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose **23**.[17]

*General procedure for the determination of the effect of cosolvents on the activity of acetylesterase*

To a suspension of 1 mL (1.16 g, 5 mmol) of glycerol triacetate in 20 ml of 0.15 N NaCl solution, the cosolvent is added in the required quantity. After the pH is adjusted to 6.5, acetylesterase (5 units) is added and the solution is stirred at room temperature. During the hydrolysis the pH is kept constant by titration with 0.02 N NaOH. The initial rate of the hydrolysis is determined during the first 120 min from the amount of added NaOH solution.

*General procedure for the acetylesterase-catalyzed hydrolyses of acetyl-protected carbohydrates*

The protected carbohydrates (0.1–1 mmol) are suspended in 350 mL of 0.15 N NaCl solution. The pH is adjusted to 6.5 with 0.02 N NaOH. Acetylesterase (5–20 units) is added and the solution is stirred at room temperature. During the hydrolysis the pH is kept constant by titration with 0.02 N NaOH. After the desired conversion is reached the reaction is stopped by freezing the solution with liquid nitrogen and subsequent lyophilization. The remaining residue is extracted three times with 150 mL of chloroform. The combined organic layers are filtered and the solvent is evaporated *in vacuo*. The resulting syrup is purified by flash chromatography using petroleum ether/ethyl acetate mixtures as eluents and the products are identified by means of their $^1$H and $^{13}$C NMR spectra.

According to this procedure the following compounds were obtained:

*1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (2)*

This compound was prepared from 0.151 g (0.5 mmol) of 3-*O*-acetyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**).Yield: 0.10 g (77 %), $R_f$ = 0.5 (ethyl acetate/petroleum ether 1:1). $^1$H-NMR (90 MHz) ($CDCl_3$): δ (ppm) = 5.95 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1-H), 4.56 (d, $J_{1,2}$ = 3.5 Hz, 1H, 2-H), 3.9-4.4 (m. 5 H, 3-H, 4-H, 5-H, 6-$H_a$, 6-$H_b$), 2.6 (s, 1H, OH), 1.55, 1.45, 1.40, 1.35 (4 s, 12H, 4 $CH_3$).

*2,3:5,6-Di-O-isopropylidene-α-D-mannofuranose (4)*

This compound was prepared from 0.302 g (1 mmol) of 1-*O*-acetyl-2,3:5,6 -di-*O*-isopropylidene -α- D-mannofuranose (**3**). Yield: 0.20 g (77 %), $R_f$ = 0.5 (ethyl acetate/*n*-hexane 1:1). $^1$H NMR (200 MHz) ($CDCl_3$): δ (ppm) = 5.38 (d, $J_{1,OH}$ = 2.5 Hz, 1H, 1-H), 4.80 (dd, $J_{2,3}$ = 6 Hz, $J_{3,4}$ = 4 Hz, 1H, 3-H), 4.61 (d, $J_{2,3}$ = 6 Hz, 1H, 2-H), 4.40 (m, 1H, 5-H), 4.18 (dd, $J_{3,4}$ = 4 Hz, $J_{4,5}$ = 7.5 Hz, 1H, 4-H), 4.05 (dd, $J_{gem}$ = 6 Hz, $J_{5,6}$ = 2Hz, 2H, 6-$H_a$, 6-$H_b$), 2.98 (d, $J_{1,OH}$ = 2.5 Hz, 1H, OH), 1.5–1.3 (4 s, 12H, 4 $CH_3$).

*2,3:4,6-Di-O-isopropylidene-β-L-sorbofuranose (6)*

This compound was prepared from 0.302 g (1 mmol) of 1-*O*- acetyl-2,3:4,6-di-*O*-isopropylidene -β- L- sorbofuranose (**5**). Yield: 0.20 g (77 %), $R_f$ = 0.5 (ethyl acetate/*n*-hexane 1:1). $^1$H-NMR (250 MHz) ($CDCl_3$): δ (ppm) = 4.48 (s, 1H), 4.32 (bs, 1H), 4.1 (bs, 1H), 4.05 (bs, 2H, 6-$H_a$, 6-$H_b$), 3.80 (m, 2H, 1-$H_a$, 1-$H_b$), 2.22 (t, $J$ = 7 Hz, 1H, OH), 1.50 (s, 3 H, $CH_3$), 1.41 (s, 3 H, $CH_3$), 1.35 (s, 6 H, 2 $CH_3$).

*Methyl 2,3-O-isopropylidene-β-D-ribofuranoside (8)*

This compound was prepared from 0.245 g (1 mmol) of methyl 5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (**7**). Yield: 0.176 g (86 %), $R_f$ = 0.35 (ethyl acetate/*n*-hexane 1:3). $^1$H NMR (250 MHz) ($CDCl_3$): δ (ppm) = 5.00 (s, 1H, 1-H), 4.86 (d, 1H, 2-H), 4.6 (d, 1H, 3-H), 4.45 (t, 1H, 4-H), 3.7 (m, 2H, 5-H, 5`-H), 3.25 (dd, 1H, OH), 3.45 (s, 3 H, $OCH_3$), 1.48 (s, 3 H, $CH_3$), 1.30 (s, 3 H, $CH_3$). $^{13}$C-NMR (100.6 MHz) $CDCl_3$): δ (ppm) = 111.9 [$C(CH_3)_2$], 109.7 (C-1), 88.1, 85.6, 81.3 (C-2, C-3, C-4), 63.7 (C-5), 55.3 ($OCH_3$), 26.2 ($CH_3$), 24.5 ($CH_3$).

*1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose (10)*

This compound was prepared from 0.151 g (0.5 mmol) of 6-*O*-acetyl-1,2:3,4-di-*O*- isopropylidene-α-D-galactopyranose (**9**). Yield: 0.95 g (73 %), $R_f$ = 0.5 (ethyl acetate/-petroleum ether 1:1). $^1$H NMR (90 MHz) ($CDCl_3$): δ (ppm) = 5.51 (d, $J_{1,2}$ = 5 Hz, 1H, 1-H), 4.66 (dd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = 2.5 Hz, 1H, 3-H), 4.36 (dd, $J_{1,2}$ = 5 Hz, $J_{2,3}$ = 2.5 Hz, 1H, 2-H), 4.26 (dd, $J_{3,4}$ = 8 Hz, $J_{4,5}$ = 1Hz, 1H, 4-H), 3.6–4.1 (m, 3 H, 5-H, 6-$H_a$, 6-$H_b$), 2.48 (s, 1H, OH), 1.5, 1.4, 1.28 (4 s, 12H, 4 $CH_3$).

Compounds **2**, **4**, **6**, **8**, and **10** were identical in all physical and spectroscopic data with the starting materials from which **1**, **3**, **5**, **7** and **9** were prepared by acetylation.

*2,3,4,6-Tetra-O-acetyl-D-glucopyranose (12)*

The tetraacetate was obtained from 0.78 g (2 mmol) of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**11**) after 20 % conversion. Yield: 0.26 g (37 %), $R_f$ = 0.65 (ethyl acetate/petroleum ether 2:1). $^1$H NMR (250 MHz) ($CDCl_3$): δ (ppm) = 5.50 (dd, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, 1H, 3-$H_\alpha$), 5.43 (d, $J_{1\alpha,2}$ = 3.5 Hz, 1H, 1-$H_\alpha$), 5.22 (dd, $J_{2,3}$ =

$J_{3,4}$ = 10 Hz, 1H, 3-Hβ), 5.07 (dd, $J_{3,4}$ = $J_{4,5}$ = 10 Hz, 1H, 4-$H_{\alpha,\beta}$), 4.88 (dd, $J_{1\alpha,2}$ = 3.5 Hz, $J_{1\beta,2}$ = 5 Hz, $J_{2,3}$ = 10 Hz, 2H, 2-$H_{\alpha,\beta}$), 4.73 (d, $J_{1\beta,2}$ = 5 Hz, 1H, 1-Hβ), 4.05–4.30 (m, 3H, 5-$H_\alpha$, 6-$H_a$, 6-$H_b$), 3.8 (m, 1H, 5-Hβ), 2.0–2.1 (4 s, 12H, 4 $COCH_3$). The NMR data are in agreement with reported values.[7]

*3,4,6-Tri-O-acetyl-D-glucopyranose (13)*

The triacetate was obtained from 0.39 g (1 mmol) of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**11**) after 40 % conversion. Yield: 0.125 g (40 %), $R_f$ = 0.35 (ethyl acetate/petroleum ether 2:1). $^1$H-NMR (400 MHz) ($CDCl_3$): δ (ppm) = 5.28 (d, $J_{1\alpha,2\alpha}$ = 3 Hz, 1H, 1-$H_\alpha$), 5.25 (dd, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, 1H, 3-$H_\alpha$), 5.06 (dd, $J_{2,3}$ = $J_{3,4}$ = 9 Hz, 1H, 3-Hβ), 4.98 (dd, $J_{4,5}$ = $J_{3,4}$ = 10 Hz, 1H, 4-$H_\alpha$), 4.91 (dd, $J_{4,5}$ = $J_{3,4}$ = 9 Hz, 1H, 4-Hβ), 4.68 (d, $J_{1,2}$ = 7.5 Hz, 1H, 1-Hβ), 4.19 (m, 4 H, 5-$H_\alpha$, 5-Hβ, 6-$H_\alpha$, 6-Hβ), 4.10 (m, 2H, 6'-$H_\alpha$, 6'-Hβ), 3.65 (dd, $J_{2\alpha,3\alpha}$ = 10 Hz, $J_{1\alpha,2\alpha}$ = 3 Hz, 1H, 2-$H_\alpha$), 3.50 (dd, $J_{2\beta,3\beta}$ = 10 Hz, $J_{1\beta,2\beta}$ = 9 Hz, 1H, 2-Hβ), 2.04–2.09 (3 s, 9 H, 3 $COCH_3$). $^{13}$C NMR (100.6 MHz) ($CDCl_3$): δ (ppm) = 171.3, 171.1, 169.9 (3 C=O), 92.1 (C-1), 73.1, 70.6, 68.1, 67.0 (C-2, C-3, C-4, C-5), 62.3 (C-6), 20.8, 20.7, 20.6 (3 $COCH_3$).

*2,3,4,6-Tetra-O-acetyl-D-galactopyranose (15)*

The tetraacetate **15** was obtained from 0.19 g (0.5 mmol) of 1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose (**14**) after 20 % conversion. Yield: 0.03 g (21 %), $R_f$ = 0.5 (ethyl acetate/petroleum ether 1:1). $^1$H NMR (250 MHz) ($CDCl_3$): δ (ppm) = 5.1–5.5 (m, 3 H, 2-H, 3-H, 4-H), 5.05 (d, $J_{1,2}$ = 4 Hz, 1H, 1-$H_a$), 4.4 (m, 1H, 5-H), 4.15 (m, 2H, 6-$H_a$, 6-$H_b$), 3.6 (bs, 1H, OH), 1.9–2.15 (4 s, 12H, 4 $COCH_3$). The NMR data are in agreement with reported values.[7]

*2,3,4,6-Tetra-O-acetyl-D-mannopyranose (17)*

The tetraacetate **17** was obtained from 0.39 g (1 mmol) of 1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose (**16**) after 20 % conversion. Yield: 0.085 g (25 %), $R_f$ = 0.5 (ethyl acetate/petroleum ether 1:1). $^1$H NMR (200 MHz) ($CDCl_3$): δ (ppm) = 5.35 (dd, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 10 Hz, 1H, 3-H), 5.23 (dd, $J_{3,4}$ = $J_{4,5}$ = 10 Hz, 1H , 4-H), 5.18 (m, 2H, 1-H, 2-H), 4.2–4.0 (m, 3 H, 5-H, 6-$H_a$, 6-$H_b$), 2.10 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$). The NMR data are in agreement with reported values.[7]

*3,6-Di-O-acetylglucal (19)*

This compound was prepared from 0.272 g (1 mmol) of 3,4,6-tri-*O*-acetylglucal (**18**). Yield: after 33 % conversion 58 mg (25 %); after 66 % conversion 15 mg (7 %), $R_f$ = 0.5 (ethyl acetate/*n*-hexane 1:1). $^1$H NMR (400 MHz) ($CDCl_3$): δ (ppm) = 6.44 (dd, $J_{1,2}$ = 6 Hz, $J_{1,3}$ = 1Hz, 1H, 1-H), 5.29 (ddd, $J_{3,4}$ = 6 Hz, $J_{3,2}$ = 2.5 Hz, $J_{1,2}$ = 1Hz, 1H, 3-H), 4.72 (dd, $J_{1,2}$ = 6 Hz, $J_{3,2}$ = 2.5 Hz, 1H, 2-H), 4.50 (dd, $J_{gem}$ = 11Hz, $J_{vic}$ = 4 Hz, 1H, 6-$H_a$), 4.38 (dd, $J_{gem}$ = 11Hz, $J_{vic}$ = 2.5 Hz, 1H, 6-$H_b$), 3.99 (ddd, $J_{4,5}$ = 8 Hz, $J_{5,6}$ = 4 Hz, $J_{5,6'}$ = 2.5 Hz, 1H, 5-H), 3.81 (ddd, $J_{4,5}$ = 8 Hz, $J_{3,4}$ = 6 Hz, $J_{4,OH}$ = 3.5 Hz, 1H, 4-H), 3.60 (d, $J_{4,OH}$ = 3.5 Hz, 1H, OH), 2.08–2.12 (2 s, 6 H, 2 $COCH_3$). The NMR data are in agreement with reported values.[11]

*4,6-Di-O-acetylglucal (20)*

This compound was prepared from 0.272 g (1 mmol) of 3,4,6-tri-*O*-acetylglucal (**18**). Yield: after 33 % conversion 22 mg (10 %); after 66 % conversion 9 mg (4 %), $R_f$ = 0.4 (ethyl acetate/*n*-hexane 1:1). $^1$H NMR (400 MHz) ($CDCl_3$): δ (ppm) = 6.40 (dd, $J_{1,2}$ = 6.5 Hz, $J_{1,3}$ = 2Hz, 1H, 1-H), 4.97 (dd, $J_{4,5}$ = 8 Hz, $J_{3,4}$ = 6 Hz, 1H, 4-H), 4.85 (dd, $J_{1,2}$ = 6.5 Hz, $J_{2,3}$ = 3.5 Hz, 1H, 2-H), 4.40 (dd, $J_{gem}$ = 11Hz, $J_{5,6}$ = 4 Hz, 1H, 6-$H_a$), 4.28 (m, 1H, 3-H), 4.22 (dd, $J_{gem}$ = 11Hz, $J_{5,6}$ = 3Hz, 1H, 6-$H_b$), 4.11 (m, 1H, 5-H), 2.59 (s, 1H, OH), 2.08–2.14 (2 s, 6 H, 2 $COCH_3$). The NMR data are in agreement with reported values.[11]

*6-O-Acetylglucal (21)*

This compound was prepared from 0.272 g (1 mmol) of 3,4,6-tri-*O*-acetylglucal (**18**). Yield: after 33 % conversion 26 mg (14 %); after 66 % conversion 70 mg (37 %), $R_f$ = 0.15 (ethyl acetate/*n*-hexane 1:1). $^1$H NMR (250 MHz) ($CDCl_3$): δ (ppm) = 6.33 (dd, $J_{1,2}$ = 6.5 Hz, $J_{1,3}$ = 2Hz, 1H, 1-H), 4.76 (dd, $J_{1,2}$ = 6.5 Hz, $J_{2,3}$ = 2Hz, 1H, 2-H), 4.66 (dd, $J_{vic}$ = 12Hz, $J_{6,5}$ = 4 Hz, 1H, 6-$H_a$), 4.2–4.4 (m, 2H, 5-H, 6-$H_b$), 3.90 (ddd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = $J_{1,3}$ = 2Hz, 1H, 3-H), 3.52 (dd, $J_{4,5}$ = 10 Hz, $J_{3,4}$ = 8 Hz, 1H, 4-H), 3.36 (s, 1H, OH), 2.30 (s, 1H, OH), 2.13 (s, 3H, $COCH_3$). The NMR data are in agreement with reported values.[18]

*Glucal (22)*

This compound was prepared as described above from 0.272 g (1 mmol) of 3,4,6-tri-*O*-acetylglucal (**18**). Yield: after 66 % conversion 50 mg (34 %), $R_f$ = 0.1 (ethyl acetate/*n*-hexane 1:1). $^1$H NMR (200 MHz) ($CD_3OD$): δ (ppm) = 6.35 (dd, $J_{1,2}$ = 6.5 Hz, $J_{1,3}$ = 2Hz, 1H, 1-H), 4.68 (dd, $J_{1,2}$ = 6.5 Hz, $J_{2,3}$ = 2Hz, 1H, 2-H), 4.31 (ddd, $J_{3,4}$ = 8 Hz, $J_{2,3}$ = $J_{1,3}$ = 2Hz, 1H, 3-H), 3.5–3.9 (m, 4 H, 4-H, 5-H, 6-$H_a$, 6-$H_b$).

*1,6-Anhydro-2,4-di-O-acetyl-β-D-glucopyranose (24)*

This compound was obtained by hydrolysis of 0.288 g (1 mmol) of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose (**23**) after 33 % conversion. Yield: 0.069 g (28 %), $R_f$ = 0.5 (ethyl acetate/*n*-hexane 2:1). $^1$H NMR (400 MHz) ($C_6D_6$): δ (ppm) = 5.49 (s, 1H, 1-H), 4.71 (s, 1H, 2-H), 4.66 (s, 1H, 4-H), 4.24 (d, $J_{5,6exo}$ = 5 Hz, 1H, 5-H), 3.95 (d, $J_{gem}$ = 7.5 Hz, 1H, 6-$H_{endo}$), 3.87 (s, 1H, 3-H), 3.56 (m, 1H, OH), 3.38 (dd, $J_{5,6}$ = 5 Hz, $J_{gem}$ = 7.5 Hz, 1H, 6-$H_{exo}$), 1.70 (s, 3H, $CH_3$), 1.63 (s, 3H, $CH_3$). The NMR data are in agreement with reported values.[13]

*1,6-Anhydro-2,3-di-O-acetyl-β-D-glucopyranose (25)*

This compound was obtained by hydrolysis of 0.288 g (1 mmol) of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose (**23**) after 33 % conversion. Yield: 0.024 g (10 %), $R_f$ = 0.3 (ethyl acetate/*n*-hexane 2:1). $^1$H NMR (400 MHz) ($CDCl_3$): δ (ppm) = 5.43 (bs, 1H, 1-H), 4.82 (*quint*, $J$ = 1.5 Hz, 1H, 3-H), 4.63 (d, $J_{2,3}$ = 1.5 Hz, 1H, 2-H), 4.59 (dd, $J_{5,6exo}$ = 6 Hz, $J_{5,6endo}$ = 1Hz, 1H, 5-H), 4.10 (dd, $J_{gem}$ = 7.5 Hz, $J_{6,5}$ = 1Hz, 1H, 6-$H_{endo}$), 3.82 (dd, $J_{gem}$ = 7.5 Hz, $J_{5,6}$ = 6 Hz, 1H, 6-$H_{exo}$), 3.58 (bs, 1H, 4-H), 2.8 (bs, 1H, OH), 2.15 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$). The NMR data are in agreement with reported values.[13]

*1,6-Anhydro-2-O-acetyl-β-D-glucopyranose (26)*

This compound was obtained by hydrolysis of 0.288 g (1 mmol) of 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose (**23**) after 33 % conversion. Yield: 0.035 g (17 %), $R_f$ = 0.15 (ethyl acetate/*n*-hexane 2:1). $^1$H NMR (250 MHz) ($CDCl_3$): δ (ppm) = 5.51 (bs, 1H, 1-H), 4.73 (bs, 1H, 2-H), 4.60 (d, $J_{5,6exo}$ = 5.5 Hz, 1H, 5-H), 4.25 (d, $J_{gem}$ = 7.5 Hz, 1H, 6-$H_{endo}$), 3.80 (dd, $J_{gem}$ = 7.5 Hz, $J_{5,6}$ = 5.5 Hz, 1H, 6-$H_{exo}$), 3.78 (m, 1H, 3-H), 3.58 (d, $J_{4,OH}$ = 4 Hz, 1H, 4-H), 2.72 (bs, 1H, OH), 2.45 (d, $J_{OH,4}$ = 4 Hz, 1H, OH), 2.15 (s, 3H, $COCH_3$). The NMR data are in agreement with reported values.[19]

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