# PORCINE LIVER ESTERASE-CATALYZED HYDROLYSIS OF METHYL TRI-*O*-ACETYL-β-D-ARABINOPYRANOSIDE, METHYL TRI-*O*-ACETYL-β-D-RIBOPYRANOSIDE AND METHYL TRI-*O*-ACETYL-β-D-RIBOFURANOSIDE

Jitka MORAVCOVÁ[1,*], Karel KEFURT[2], Romana HLADŮVKOVÁ and Jan STANĚK[3]

*Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Prague, Technická 5, 166 28 Prague, Czech Republic; e-mail: [1] jitka.moravcova@vscht.cz, [2] karel.kefurt@vscht.cz, [3] jan.stanek@vscht.cz*

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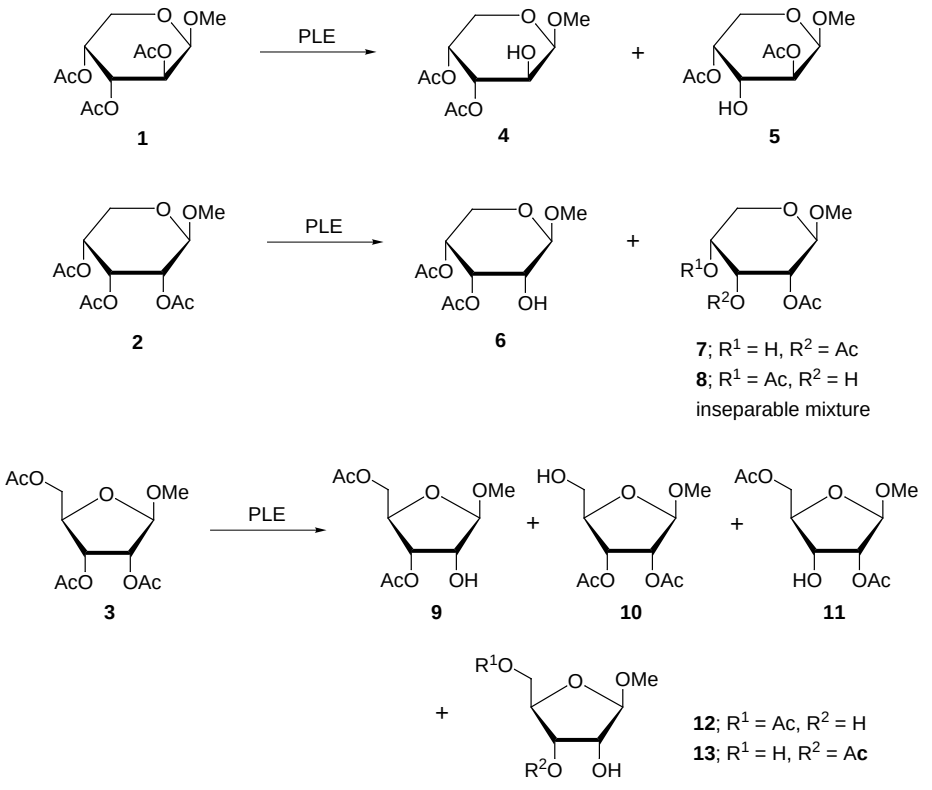
Methyl 2,3,4-tri-*O*-acetyl-β-D-arabinopyranoside (**1**), methyl 2,3,4-tri-*O*-acetyl-β-D-ribopyranoside (**2**), and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**3**) were deacetylated in porcine liver esterase-catalyzed reactions. Triacetate **1** gave methyl 3,4-di-*O*-acetyl-β-D-arabinopyranoside in 70% preparative yield while the regioselectivities found for the substrates **2** and **3** were substantially lower. Both the Michaelis constant and maximum rate were calculated for deacetylation of **1**, **2**, and **3**. The results were interpreted using an active site model for the esterase proposed by Jones.

**Key words**: Carbohydrates; Esters; Hydrolases; Biocatalysis; Deacetylation; Enzyme catalysis; Hydrolyses; Protecting groups.

At present, the enzyme-catalyzed hydrolysis of esters or the reverse process, *i.e.* esterification, using commercially available lipases or esterases forms about 40% of the biotransformations[1]. Porcine liver esterase (PLE, E.C. 3.1.1.1) has been widely utilized[2] for the manipulations of acetyl protecting groups in the carbohydrate chemistry. In continuing our studies[3–5], the regioselectivity of PLE-catalyzed hydrolysis of three methyl tri-*O*-acetyl-β-D-glycosides was investigated. Of particular interest in the study described here is the evaluation of applicability of Jones's model[6] of a PLE active site.

## RESULTS AND DISCUSSION

The course of the PLE-catalyzed hydrolyses (Scheme 1) of methyl 2,3,4-tri-*O*-acetyl-β-D-arabinopyranoside (**1**), methyl 2,3,4-tri-*O*-acetyl-β-D-ribopyranoside (**2**), and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (**3**) were monitored by GLC (Table I).




SCHEME 1

Triacetate **1** gave a mixture of two diacetates **4** and **5** in the ratio of 6.9 : 1, which dropped to 2.1 : 1 during the reaction. When the PLE hydrolysis of **1** was carried out on preparative scale the predominant crystalline methyl 3,4-di-*O*-acetyl-β-D-arabinopyranoside (**4**) was isolated in 59% yield. $^1$H and $^{13}$C chemical shifts of diacetate **4** and vicinal coupling constants (Table II) confirm its structure. In the $^1$H NMR spectrum, the signal of H-2 is shifted upfield in comparison with that of triacetate **1** ($\Delta\delta$ 1.21 ppm) and it shows a large *J*(2,OH) coupling (11 Hz). The respective OH-2 resonance occurs as a doublet at 2.03 ppm. Furthermore, the high *J*(2,OH) coupling indicates a fixed *anti*-periplanar orientation of both H-2 and H of the hydroxy group. The strong association of OH-2 group into intramolecular hydrogen bonding with O-1 atom is evidenced from the IR spectrum exhibiting absorption at 3 579 cm$^{-1}$ while the free secondary hydroxy group gave[7] a band at 3 630 cm$^{-1}$. The chemical shifts of H-1 and H-2 atoms (Table II) are in accord with partial NMR data of an inpure **4** published previously[8].

Based on the vicinal coupling constants (Table II), the preferential $^1C_4$(D) conformation is proposed for methyl 3,4-di-*O*-acetyl-β-D-arabinopyranoside (**4**). The minor diacetate **5** was not isolated in a pure state, but the inspection of $^1$H NMR data allowed assigning its structure to methyl 2,4-di-*O*-acetyl-β-D-arabinopyranoside. Evidence for the presence of OH-3 was found by a comparison of the chemical shift of H-3 for **5** and **1** (see Experimental), the former being shifted upfield (Δδ 1.22 ppm). Additional support came also from multiplicity of the H-3 signal of 2,4-diacetate **5** indicating *J*(3,OH) coupling of 7.3 Hz.

In an attempt to increase the preparative yield of the novel diacetate **4**, the reaction conditions were carefully optimized. After 3.5 h (pH 7.0, 20 °C) incubation of **1** with PLE, 3,4-diacetate **4** could be finally obtained in the 70% isolated yield. Thus, the 2-*O*-acetyl group in triacetate **1** is hydrolyzed more rapidly than the corresponding 3-*O*-acetyl group. Interestingly, the presence of the third possible diacetate was not traced within the experimental error. This means that PLE leaves the 4-*O*-acetyl group in **1** intact or subsequent hydrolysis of the 2,3-diacetate possibly formed is extremely fast.

The PLE hydrolysis of *ribo*-triacetate **2** was slower than that of *arabino*-triacetate **1** (Table I). In the initial stage of the reaction, the GLC analysis revealed the occurrence of all three possible diacetates of methyl β-D-ribopyranoside **6**–**8** in the 1.6 : 1 : 1.6 ratio suggesting a low regioselectivity of the biotransformation. After consumption of 1.2 equivalents of sodium hydroxide, the relative distribution of these diacetates changed giving the 1.2 : 1 : 2.1 ratio. However, when a preparative experiment un-

TABLE I
The composition[a] (in %) of the reaction mixtures in the PLE hydrolysis (pH 8.0, 35 °C) of triacetates **1**, **2**, and **3**

| Conversion[b] | **1** | | | **2** | | | **3** | | |
|---|---|---|---|---|---|---|---|---|---|
| | tri- | di- | mono- | tri- | di- | mono- | di- | tri- | mono- |
| 0.6 | 39.4 | 51.0 | 9.6 | 46.4 | 49.7 | 3.9 | 0[c] | 59.7[c] | 40.3[c] |
| 1.2 | 4.7 | 75.6 | 19.7 | 6.9 | 48.0 | 15.1 | 0 | 45.9 | 54.1 |
| 1.8 | 0 | 32.5 | 67.5 | 0 | 54.7 | 45.3 | 0 | 35.1 | 64.9 |

[a] The response factors were neglected; [b] expressed as the consumption of NaOH equivalents; [c] consumption of NaOH equivalents = 1.0

TABLE II
NMR data for compounds **4**, **6**, and **9**–**11**

| Parameter | **4** | **6** | **9** | **10** | **11** |
|---|---|---|---|---|---|
| | | | Chemical shifts (δ, ppm) | | |
| H-1 | 4.85 | 4.75 | 4.88 | 4.92 | 4.87 |
| H-2 | 3.98 | 3.77 | 4.28 | 5.24 | 5.02 |
| H-3 | 5.13 | 5.22 | 5.13 | 5.36 | 4.32 |
| H-4 | 5.26 | 5.22 | 4.25–4.45 | 4.23 | 4.30–4.45 |
| H-5 | 3.88 | 3.96 | 4.35 | [a] | 4.40 |
| H-5′ | 3.68 | 3.77 | 4.13 | [a] | 4.10 |
| $CH_3O$ | 3.45 | 3.44 | 3.38 | 3.44 | 3.36 |
| $CH_3CO$ | 2.15, 2.08 | 2.16, 2.11 | 2.15, 2.10 | 2.12, 2.07 | 2.16, 2.11 |
| OH | 2.03 | 2.81 | 2.51 | 2.40 | 2.47 |
| C-1 | 100.01 | 101.43 | 108.56 | 106.53 | 106.00 |
| C-2 | 70.33[b] | 68.64[b] | 78.60 | 82.42 | 80.83 |
| C-3 | 69.39[b] | 68.64[b] | 76.70[b] | 75.27[b] | 74.10[b] |
| C-4 | 67.50[b] | 66.50[b] | 71.26[b] | 71.26[b] | 71.26[b] |
| C-5 | 60.67 | 60.45 | 64.85 | 62.93 | 64.85 |
| $CH_3O$ | 55.39 | 55.34 | 55.29 | 55.76 | 55.16 |
| $CH_3CO$ | 20.82, 20.77 | 20.89, 20.72 | 20.5–20.9 | 20.5–20.9 | 20.50–20.90 |
| $CH_3CO$ | 169.74, 169.30 | 168.96, 168.61 | 170.1–171.0 | 170.1–171.1 | 170.1–171.0 |
| | | | Vicinal coupling constants ($J$(H,H), Hz) | | |
| $J$(1,2) | 3.7 | 3.5 | 0.5 | 1.0 | 0 |
| $J$(2,3) | 10.3 | 4.2 | 4.4 | 5.1 | 4.8 |
| $J$(3,4) | 3.5 | 3.7 | 6.1 | 5.9 | 5.2 |
| $J$(4,5) | 1.0 | 1.7 | 5.4 | 3.9 | 2.8 |
| $J$(4,5′) | 2.1 | 1.8 | 5.7 | 3.9 | 5.7 |
| $J$(5,5′) | 13.0 | 13.0 | 12.3 | [a] | 11.4 |
| $J$(H,OH) | 11.0 | 7.3 | 4.4 | 3.8, 7.6 | 6.6 |

[a] Chemical shift cannot be determined; [b] these values may be interchanged.

der these experimental conditions was performed, the isolated fraction contained diacetates **6**–**8** in a slightly different ratio, 2.1 : 1 : 1.2, as checked by GLC. In this respect, either the acetyl migration of *cis*-arranged acetoxy and hydroxy groups during the work-up or the different extraction efficiency has to be taken into account. Diacetate **6** was the only pure product isolated in 20% yield from a complex reaction mixture. The interpretation of both $^{1}$H and $^{13}$C NMR data (Table II) confirmed the structure of methyl 3,4-di-*O*-acetyl-β-D-ribopyranoside for **6**. Based on the relatively low vicinal coupling constants $J(1,2)$, $J(4,5')$, and $J(4,5)$ (Table II), the preferential $^{1}C_{4}$(D) conformation of 3,4-diacetate **6** can be proposed although it dictates an unfavorable diaxial interaction between the substituents at C-2 and C-4 atoms. The association of OH-2 into the intramolecular hydrogen bonding ($\nu$ 3 587 cm$^{-1}$) can probably stabilize this conformation.

The rate of the PLE hydrolysis of tri-*O*-acetylribofuranoside **3** was comparable with that of triacetate **1** (Table I). The high regioselectivity of PLE attack resulted in a substantial excess of diacetate **9** over diacetate **10** (ratio 68 : 1 in the initial stage of reaction) according to GLC. GC/MS analysis was then carried out giving the two resolved peaks with retention times 9.37 and 10.23 min. Both peaks can be attributed to diacetates (MW 248) due to the presence of a fragment at *m/z* 217 (M – 31). The mass spectral fragmentation pattern of diacetate **10** with a shorter retention time corresponds with the data reported[9] for methyl 2,3-di-*O*-acetyl-β-D-ribofuranoside. The assignment of **9** ($t_R$ 10.23 min) to methyl 3,5-di-*O*-acetyl-β-D-ribofuranoside was based on its abundant fragment at *m/z* 175 absent from the spectrum of **10** (see Experimental). This fragment can be identified as $C_7H_{12}O_5$ arising from the molecular ion by a splitting of both C-1:O-4 and C-2:C-3 bonds. The presence of the third possible isomer, methyl 2,5-di-*O*-acetyl-

TABLE III
Michaelis constants and maximal rates calculated for triacetates **1**–**3**

| Compound | Correlation coefficient | $K_M \cdot 10^3$ mol dm$^{-3}$ | $V_{max} \cdot 10^4$ mol dm$^{-3}$ s$^{-1}$ |
|---|---|---|---|
| **1** | 0.9994 | 6.93 | 3.39 |
| **2** | 0.9991 | 10.08 | 2.06 |
| **3** | 0.9992 | 3.57 | 3.18 |

β-D-ribofuranoside (**11**), was not observed within the experimental error. Furthermore, GC/MS analysis showed another two peaks at 8.26 (major) and 9.07 min displaying the highest fragment at *m/z* 175. If the elimination of methoxy radical from the molecular ion is considered, molecular weight 206 corresponds to monoacetates **12** and **13**. The fragmentation pathway of minor monoacetate **13** matches closely the published mass spectrum of methyl 5-*O*-acetyl-β-D-ribofuranoside[9]. The most abundant monoacetate **12** could be methyl 3-*O*-acetyl-β-D-ribofuranoside rather than 2-*O*-acetyl isomer due to similar fragmentation patterns of **12** and 3,5-diacetate **9** (see Experimental).

Although GLC checking assessed the 3,5-diacetate **9** as a predominant product, the preparative experiment on PLE hydrolysis of triacetate **3** led, after chromatography, to a mixture of all three diacetates **9**–**11** in 76% yield. On the basis of relative intensities of the respective O-$CH_3$ signals in $^1$H NMR spectrum, the 8 : 1 : 10 ratio (**9** : **10** : **11**) was estimated. Because of only partial overlapping of $^1$H and $^{13}$C resonances, the NMR data of each of diacetates **9**–**11** were extracted (Table II). The observed $^1$H and $^{13}$C shifts of 2,3-diacetate **10** were close to those reported previously[9]. Compared with H-2 of triacetate **3** (δ 5.23 ppm), the H-2 shift for **9** (Table II) experiences a shielding effect (Δδ 0.95 ppm) associated with the presence of hydroxy at C-2 of **9**. This finding is consistent with the proposed structure of methyl 3,5-di-*O*-acetyl-β-D-ribofuranoside for **9**. Similarly, compared with H-3 of triacetate **3** (δ 5.33 ppm), H-3 of **11** is also shielded (Δδ 1.05 ppm) and thus the structure of methyl 2,5-di-*O*-acetyl-β-D-ribofuranoside can be confirmed for **11**.

The ratio of monoacetates **12** and **13** obtained by preparative liquid chromatography was also quite different from that obrained by GS/MS of the crude mixture, 5-*O*-acetate **13** being the predominant product. Thus, the course of the PLE hydrolysis of triacetate **3** investigated by GLC was also inconsistent with the actual outcome from the preparative scale experiment. These findings again suggest the occurrence of acetyl migration during the subsequent isolation and separation procedures. Acetyl migration in partially acetylated polyhydroxy compounds is well known in both acid and alkaline media[10].

The determination of the Michaelis constant ($K_M$) and the maximum rate ($V_{max}$) is a convenient approach to the study of enzymic reactions. Thus, the PLE hydrolyses of **1**–**3** with five different initial concentrations were followed and corresponding initial velocities were calculated. A Lineweaver–Burk equation was then applied to calculate both $K_M$ and $V_{max}$ (Table III).

The $K_M$ values of acetates **1**–**3** are approximately one fifth of the $K_M$ found[4,5] for methyl di-*O*-acetyl-5-deoxypentofuranosides indicating a stronger binding in enzyme–substrate (ES) complexes.

The decreasing affinity of model triacetates to PLE in the order **3** > **1** > **2** was accompanied with the lowest $V_{max}$ found for **2**. This finding is in agreement with the kinetic study in which the PLE hydrolysis of **2** is the slowest reaction. Nevertheless, all the substrates **1**–**3** were hydrolyzed more rapidly than methyl di-*O*-acetyl-5-deoxypentofuranosides[4,5].

Finally, we attempted to interpret the regioselectivity of PLE hydrolyses of **1**–**3** using a generally applicable active site model[6] for PLE combined with additional criteria[4,5]. For triacetate **1**, an ES complex leading to hydrolysis of 4-*O*-acetyl group is precluded since any of remaining *O*-acetyl groups cannot adopt the required accommodation into a PLE active site. On the contrary, if the 2-*O*-acetyl group is located in the catalytic serine region of PLE, the nonhydrolyzed 3-*O*-acetyl group accommodates fully in a favored orientation. Simultaneously, both methoxy group at C-1 and 4-*O*-acetyl group stabilize the ES complex due to the favorable interactions with polar and hydrophobic cavities of PLE. The reversed binding of acetoxy groups located at C-2 and C-3 leading to the hydrolysis of **1** at position 3 is also possible, but the corresponding ES complex is substantially less stable because of a lower effect of the remaining substituents. Thus the analysis using the Jones active site model predicts a high regioselectivity of PLE hydrolysis of triacetate **1** resulting in an excess of 3,4-diacetate **4**. This assumption is in total agreement with the real course of the hydrolysis of **1** as mentioned above.

The *ribo*-triacetate **2** displays the opposite configuration at C-2 compared with **1** and this difference leads to total loss of regioselectivity. Moreover, triacetate **2** was the worst PLE substrate with the lowest reaction rate. This means that all three possible PLE-**2** complexes appear energetically discriminated and essentially equivalent. In this case, all PLE complexes considered for **2** are characterized by inconvenient orientation of nonhydrolyzed *O*-acetyl group directed away from an optimum fit due to the relative *cis* configuration at C-2, C-3, and C-4. Nevertheless, the fact that PLE-**2** complexes exist should be ascribed to at least partial interaction of either 1-methoxy group or C-5 methylene group with a hydrophobic pocket of PLE.

Taking into account the published exclusive PLE hydrolysis[11] of methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside in position 4, some guidelines covering a behavior of methyl 2,3,4-tri-*O*-acetylpentopyranoside towards the PLE attack could be proposed. Thus, the selectivity of PLE hydrolysis is controlled by the relative configuration of *O*-acetyl groups. The most important

requirement is the *trans* orientation of any two vicinal *O*-acetyl groups leading to the favored fit of the substrate into the PLE active site[6]. In this case, PLE does not distinguish between the absolute configurations of C-2, C-3, and C-4.

The situation becomes more evident, if the exclusive hydrolysis of tri-*O*-acetylribofuranoside **3** in position 2 is taken into account. The C-4 exocyclic substituent in **3** must play a decisive role because it can fully occupy the hydrophobic pocket of PLE allowing a strong interaction. In opposite, a competitive PLE-**3** complex for the hydrolysis in position 3 is completely discriminated because it leaves this pocket empty.

In conclusion, our results suggest that the Jones active site model proposed for PLE can be a useful tool for the prediction of selectivity of enzymic hydrolysis of pentoside triacetates. Excellent regioselectivity was achieved with PLE hydrolysis of methyl 2,3,4-tri-*O*-acetyl-β-D-arabinopyranoside, methyl 3,4-di-*O*-acetyl-β-D-arabinopyranoside was readily obtained in 70% isolated yield.

## EXPERIMENTAL

Optical rotations were measured on a JASCO Model DIP-370 polarimeter and are given in $10^{-1}$ deg cm$^2$ g$^{-1}$. Melting points were determined with a Kofler hot block and are uncorrected. NMR data were extracted from spectra measured in $CDCl_3$ solutions (tetramethylsilane as an internal standard) at 25 °C with a Bruker AM 400 spectrometer ($^1$H, 400 MHz; $^{13}$C, 100.62 MHz). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Mass spectra were recorded on a Jeol DX 303 instrument coupled with a SE-54 silica capillary column (30 m × 0.32 mm i.d., film thickness 1.0 μm) using EI technique at 70 eV. IR spectra of solutions in $CCl_4$ were measured on a FT IR Nicolet 740 spectrometer. The enzyme-catalyzed hydrolyses were carried out under nitrogen in a pH-stat RTS 822 (Radiometer, Denmark) using thermostated vessels with magnetic stirrer. Porcine live esterase (PLE) was purchased from Sigma (U.S.A.) as a suspension in 3.2 M $(NH_4)_2SO_4$, containing 8 mg of protein/ml. Column chromatography was performed on silica gel 100–160 μm (Lachema, Czech Republic), and TLC on silica gel according to Stahl (10–40 μm, Merck, Germany). The spots on TLC were detected by spraying with 1% $Ce(SO_4)_2$ in 10% $H_2SO_4$ and subsequent mineralization. Solutions were concentrated under reduced pressure with a bath temperature below 40 °C.

*GLC*. Analyses were performed with a Hewlett–Packard 5890 A instrument equipped with a flame-ionization detector. A fused silica capillary column (50 m × 0.32 mm i.d.) with chemically bonded methyl (phenyl) silicone (5%, film thickness 0.5 μm) was used with nitrogen as a carrier gas at a flow rate 1.8 ml/min (split 1 : 50). Temperature: 130 °C (1 min), 4 °C/min up to 220 °C, 60 °C/min up to 280 °C, 280 °C (1 min); detector, 230 °C; injector, 200 °C. The following retention times (in min) were obtained: **1**, 17.57; **4**, 14.93; **5**, 15.37; methyl *O*-acetyl-β-D-arabinopyranosides, 12.26 and 12.50; methyl β-D-arabinopyranoside, 7.29; **2**, 18.21; **6**, 14.87; methyl di-*O*-acetyl-β-D-ribopyranosides, 14.39 and 16.05; methyl

*O*-acetyl-β-D-ribopyranosides, 11.41, 11.61, and 12.82; methyl β-D-ribopyranoside, 8.40; **3**, 18.02; **9**, 15.83; **10**, 14.89; **12**, 12.12; **13**, 12.32; methyl β-D-ribofuranoside, 6.81.

*Determination of PLE activity*. To a mixture of ethyl acetate (50 μl) in 0.5 M KCl (2 ml) at 20, 25, 30 or 35 °C, a suspension of PLE (10 μl of original concentrate) was added. The consumption of the 0.103 M NaOH solution necessary to maintain pH 8.0 was measured during 10 min. The activity of PLE was calculated from the rate of hydrolysis and expressed as U/mg of protein (1 U hydrolyses 1 μmol of ethyl acetate per min); U/°C: 47.4/20, 70.3/25, 89.9/30, 114.8/35.

*Determination of $K_M$ and $V_{max}$*. A solution of substrate **1**–**4** (0.004–0.08 mmol) in 0.5 M KCl (2 ml) at 35 °C and pH 8.0 was stirred under nitrogen for 10 min to monitor spontaneous hydrolysis. Then a suspension of PLE (10 μl of the original concentrate) was added and stirring was continued. An initial time-dependence of consumption of 0.103 M NaOH necessary to maintain pH 8.0 was measured during the first 10 min. The initial velocity was then calculated as a slope of linear dependence of molar concentration of acetic acid formed and reaction time during the first minute of the reaction.

*PLE-Catalyzed reaction monitored by GLC*. To a 0.035 M solution (2 ml) of each substrate **1**–**3** in 0.5 M KCl at 35 °C under nitrogen atmosphere, after adjustment of pH to 8.0 and stirring for 10 min, PLE (10 μl of the original concentrate) was added. By titration with 0.103 M NaOH, pH of the solution was kept at 8.0. Aliquots of the reaction mixture (200 μl) were withdrawn during several hours and the deacetylation stopped by addition of toluene (100 μl). Each sample was concentrated to dryness, the residue was diluted with ethanol (100 μl), and the supernatant was directly analyzed by GLC to give the molar ratios of the starting compound and various deacetylation products.

Methyl 2,3,4-Tri-*O*-acetyl-β-D-arabinopyranoside (**1**)

Triacetate **1** was obtained by the known procedure[12], m.p. 74–77 °C, ref.[12] m.p. 75–77 °C; $[\alpha]_D^{20}$ –186 (*c* 1, $CHCl_3$), ref.[12] $[\alpha]_D^{20}$ –187 (*c* 1, $CHCl_3$). $^1$H NMR was in agreement with this already published[12]. $^{13}$C NMR: 97.00 (C-1); 68.80 (C-3); 68.02 (C-2); 66.75 (C-4); 59.89 (C-5); 55.20 ($CH_3O$); 20.81, 20.70, 20.57 (3 × $CH_3CO$), 169.34, 169.32, 168.92 (3 × $CH_3CO$).

Methyl 2,3,4-Tri-*O*-acetyl-β-D-ribopyranoside (**2**)
and Methyl 2,3,5-Tri-*O*-acetyl-β-D-ribofuranoside (**3**)

D-Ribose (3.2 g, 22.8 mmol) was stirred with 1% HCl in MeOH (32 ml) under reflux for 6.5 h. The acid was then neutralized with solid $Ag_2O$, the solids were filtered off and washed with MeOH. The combined filtrates were concentrated in vacuum. The residue (3.7 g) was dissolved in pyridine (5 ml) and acetic anhydride (14 ml) and the solution was kept at room temperature. After 6 h, the mixture was decomposed with water, concentrated to dryness, and separated by flash chromatography on silica gel (100 g) with diethyl ether–petroleum ether (1 : 1).

*Methyl 2,3,5-tri-O-acetyl-β-D-ribofuranoside* (**3**) (1.1 g, 18%, $R_F$ 0.38); $[\alpha]_D^{20}$ +14 (*c* 1, MeOH), ref.[13] $[\alpha]_D^{20}$ +14.6 (*c* 1, MeOH). $^1$H NMR was identical to this reported[14]. $^{13}$C NMR: 105.64 (C-1); 78.13, 74.24, 71.15 (C-2, C-3, C-4); 64.06 (C-5); 54.95 ($CH_3O$); 20.65, 20.46, 20.38 (3 × **C**$H_3$CO); 169.59, 168.63, 168.60 (3 × $CH_3$**C**O).

*Methyl 2,3,4-tri-O-acetyl-β-D-ribopyranoside* (**2**) (2.9 g, 50.4%, $R_F$ 0.28); $[\alpha]_D^{20}$ –87 (*c* 1, $CHCl_3$), ref.[12] $[\alpha]_D^{20}$ –88 (*c* 1, $CHCl_3$). $^1$H NMR: 5.37 dd, 1 H, *J*(2,3) = 3.5, *J*(3,4) = 3.2 (H-3); 5.16 ddd, 1 H, *J*(4,5) = 2.7, *J*(4,5′) = 4.0 (H-4); 5.03 dd, 1 H (H-2); 4.72 d, 1H, *J*(1,2) = 3.3 (H-1); 3.99 dd,

1 H (H-5); 3.80 dd, 1 H, *J*(5,5′) = 12.6 (H-5′); 3.44 s, 3 H ($CH_3O$); 2.05 s, 2.14 s, 2.15 s, each 3 H (3 × $CH_3CO$); ref.[14] gave $^1$H NMR (benzene-$d_6$): 5.69 dd, 1 H, *J*(2,3) = 10.86, *J*(3,4) = 3.42 (H-3); 5.57 dd, 1 H (H-2); 5.42 ddd, 1 H, *J*(4,5) = 1.44, *J*(4,5′) = 1.98 (H-4); 5.05 d, 1 H, *J*(1,2) = 3.43 (H-1); 3.39 dd, 1 H (H-5); 3.345 dd, 1 H, *J*(5,5′) = 13.00 (H-5′); 2.96 s, 3 H ($CH_3O$); 1.75 s, 1.66 s, 1.65 s, each 3 H (3 × $CH_3CO$). $^{13}$C NMR corresponded with literature data[15].

Finally, methyl 2,3,4-tri-*O*-acetyl-α-D-ribopyranoside (1.8 g, $R_F$ 0.20) contamined with **2** was obtained.

Preparative PLE Hydrolysis. General Procedure

A suspension of each substrate **1**–**3** (200 mg, 0.668 mmol) in phosphate buffer (6 ml, pH 7.8) at 35 °C was stirred for 10 min to monitor a spontaneous hydrolysis. Then the suspension of PLE (50 µl of the original concentrate) was added and stirring was continued. By titration with 0.103 M NaOH the pH of reaction mixture was kept at 8.0 and reaction was monitored by TLC. After consumption of 1 equivalent of NaOH (substrate/min: **1**/43, **2**/200, **3**/38), the reaction was stopped by toluene (1 ml) and evaporated to dryness. A residue was extracted with diethyl ether (3 × 50 ml), the combined filtrates were dried with $MgSO_4$, and solvent was evaporated.

*Preparative hydrolysis of methyl 2,3,4-tri-O-acetyl-*β*-D-arabinopyranoside* (**1**). The residue (155 mg) was separated on silica gel (50 g) in toluene–ethanol (95 : 5). The first eluted compound (20 mg, $R_F$ 0.47, benzene–ethanol (95 : 5)) was identical with the incoming **1**. Further elution gave diacetate **4** (60 mg, $R_F$ 0.32) and a mixture of both diacetates **4** and **5** (76 mg), which yielded after chromatography a further portion of **4** (40 mg). Thus, the overall yield of methyl 3,4-di-*O*-acetyl-β-D-arabinopyranoside (**4**) was 59%, m.p. 136–138 °C; $[\alpha]_D^{20}$ –220 (*c* 1, $CHCl_3$). $^1$H and $^{13}$C NMR data are collected in Table II. For $C_{10}H_{16}O_7$ (248.2) calculated: 48.38% C, 6.50% H; found: 48.69% C, 6.61% H. 2,4-Diacetate **5** (16 mg, $R_F$ 0.31) was not purified and its constitution was only deduced from $^1$H NMR data. $^1$H NMR: 5.16 m, 1 H (H-4); 5.05 dd, 1 H, *J*(2,3) = 10.3 (H-2); 4.90 d, 1 H, *J*(1,2) = 3.5 (H-1); 4.16 m, 1 H, *J*(3,OH) = 7.3, *J*(3,4) = 3.7 (H-3); 3.83 dd, 1 H, *J*(4,5) = 1.0 (H-5); 3.76 dd, 1 H, *J*(4,5′) = 1.8, *J*(5,5′) = 13.1 (H-5′); 2.18 s and 2.16 s, each 3 H (2 × $CH_3CO$); 3.40 s, 3 H ($CH_3$); 2.36 d, 1 H (3-OH).

*Preparative hydrolysis of methyl 2,3,4-tri-O-acetyl-*β*-D-ribopyranoside* (**2**). The residue (120 mg) was separated as described above. Chromatography recovered the unreacted **2** (10 mg, $R_F$ 0.42, benzene–ethanol (95 : 5)) followed by diacetate **6** (15 mg, $R_F$ 0.29) and a mixture of all diacetates **6**–**8** (65 mg, $R_F$ 0.29, 0.26, 0.23). Re-chromatography of the later fraction gave pure diacetate **6** (20 mg), the overall yield of syrupy methyl 3,4-di-*O*-acetyl-β-D-ribopyranoside (**6**) reached 20%; $[\alpha]_D^{20}$ –113 (*c* 1.2, $CHCl_3$). For $C_{10}H_{16}O_7$ (248.2) calculated: 48.38% C, 6.50% H; found: 48.83% C, 6.63% H. Finally, an unseparable mixture of monoacetyl derivatives (25 mg, $R_F$ 0.09) was eluted.

*Preparative hydrolysis of methyl 2,3,5-tri-O-acetyl-*β*-D-ribofuranoside* (**3**). The residue (150 mg) was separated as described above. Chromatography recovered unreacted **3** (10 mg, $R_F$ 0.51, benzene–ethanol (95 : 5)) followed by an unseparable mixture of diacetates **9**–**11** (130 mg, 76%, $R_F$ 0.27). $^1$H and $^{13}$C NMR data are collected in Table II. 3,5-Diacetate **9**, MS (*m/z*): 247 (0.3), 230 (0.8), 217 (3.5), 188 (2), 175 (40), 157 (6), 128 (20), 115 (100), 103 (28), 99 (20), 86 (62), 73 (33), 68 (50), 59 (30), 54 (36). 2,3-Diacetate **10**, MS (*m/z*): 217 (32), 157 (16), 128 (8), 115 (100), 103 (6), 85 (24), 73 (10), 69 (16), 54 (8). Further elution yielded monoacetates **12** and **13** (10 mg, $R_F$ 0.09, benzene–5% ethanol) as an unseparable mixture. For **12**, MS (*m/z*): 175 (3), 146 (6), 133 (23), 115 (15), 103 (37), 86 (93), 73 (93), 59 (63), 54 (100). For

**13**, MS (*m/z*): 175 (43), 157 (3), 142 (3), 133 (3), 115 (100), 103 (10), 87 (33), 73 (50), 68 (18), 59 (30), 54 (43) corresponded with literature data[9].

Optimized Preparative PLE Hydrolysis of **1**

A suspension of substrate **1** (720 mg, 2.4 mmol) in phosphate buffer (6 ml, pH 7.8) at 20 °C was stirred for 10 min to monitor a spontaneous hydrolysis. Then a suspension of PLE (150 µl of the original concentrate) was added and stirring was continued. By titration with 0.103 M NaOH the pH of reaction mixture was kept at 7.0 and the reaction was monitored by TLC. After consumption of 1 equivalent of NaOH (3.5 h), the reaction was stopped with toluene (2 ml) and evaporated to dryness. The residue was extracted with diethyl ether (3 × 100 ml), the combined filtrates were dried with anhydrous $MgSO_4$ and the solvent was evaporated. Flash chromatography of the residue (510 mg) yielded unreacted **1** (30 mg), diacetate **4** (438 mg, 71%), and diacetate **5** (20 mg) contamined by **4**. The spectral characterization, m.p., and optical rotation of **4** were identical to those reported above.

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