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The acetates of *p*-nitrophenyl α-L-arabinofuranoside—Regioselective preparation by action of lipases

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Abstract—All possible di-*O*-acetates and mono-*O*-acetates of *p*-nitrophenyl α-L-arabinofuranoside were prepared by chemoenzymatic way using lipases. The 2,3-di-*O*-acetate was obtained in 90% yield by deacetylation of the primary acetyl group of per-*O*-acetylated *p*-nitrophenyl α-L-arabinofuranoside by *Candida cylindracea* lipase (CCL) or *Candida rugosa* lipase (LAY). The 2,5- and 3,5-di-*O*-acetates were obtained by acetylation of *p*-nitrophenyl α-L-arabinofuranoside by *Pseudomonas cepacia* lipase (LPS-30) in organic solvents. The 5-*O*-acetate was regioselectively synthesised in 95% yield by acetylation of *p*-nitrophenyl α-L-arabinofuranoside catalysed by porcine pancreas lipase. Finally, the 2- and 3-*O*-acetates of *p*-nitrophenyl α-L-arabinofuranoside were obtained in two steps. The enzymatic di-*O*-acetylation of *p*-nitrophenyl α-L-arabinofuranoside by LPS-30 was followed by enzymatic hydrolysis of the primary acetyl group by CCL or LAY.

1. Introduction

Development of economically feasible and ecologically friendly processes of bioconversion of renewable plant biomass requires a detailed knowledge of catalytic properties of microbial glycosyl hydrolases and esterases involved in biodegradation of plant cell walls. In connection with this effort there is an increasing demand for a variety of regioselectively acetylated *p*-nitrophenyl glycosides[1] that can serve as precursors for chromogenic substrates important for investigation of catalytic properties of the enzymes and for elaboration of simple methods for enzyme monitoring.[2]

α-L-Arabinofuranosyl residues are widely distributed in plant cell-wall polysaccharides.[3] These polymers[4,5] and enzymes hydrolysing their glycosidic linkages, such as α-L-arabinofuranosidases,[6] are gaining increasing attention. Recently, a few reports reported on transglycosylation reactions of α-L-arabinofuranosidases leading to alkyl arabinofuranosides[7] or α-L-arabinofuranose-containing disaccharides.[8]

The problem of selective modification of carbohydrates as polyhydroxylated molecules is generally known. Different types of multi-step chemical protection/deprotection strategies had to be developed for structurally different kinds of saccharides to accomplish reactions at selected positions. Several approaches have also been introduced in the case of α-L-arabinofuranosides.[4,5,9,10]

In order to shorten the lengthy protection/deprotection sequences associated with the modification of monosaccharides, we turned our attention to enzymes that can selectively modify carbohydrates. Lipases are well-known biocatalysts widely used for regioselective deacylation or acylation of carbohydrates.[11–17] Most of the enzymatic studies have focused on hexopyranose derivatives. Modifications of furanose derivatives by lipases,[11,18–22] esterases[23–25] or proteases[26–29] concern mostly the primary position of furanoside ring. Exceptions are some D-furanosides[11,18,21,23–25] or ribonucleosides.[20,26]

Recently we synthesised 2-*O*- and 5-*O*-feruloylated *p*-nitrophenyl α-L-arabinofuranoside[30] as substrates for feruloyl esterases.[31] The starting di-*O*-acetates in this

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synthesis were regioselectively prepared trough enzymatic steps. Here, we report summary of the optimised enzymatic synthesis of three possible di-*O*-acetates and three mono-*O*-acetates of *p*-nitrophenyl α-L-arabinofuranoside. Regioselective acetylation or deacetylation was accomplished by transesterification in organic media or hydrolysis catalysed by commercial lipases. We did not find any paper, except our preliminary work,[30] dealing with enzymatic acylation or hydrolysis of L-arabinofuranoside derivatives.

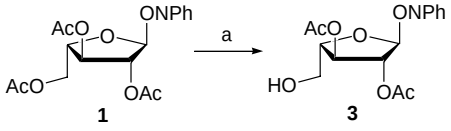
2. Results and discussion

2.1. Preparation of *p*-nitrophenyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside (3)

The starting compound *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-arabinofuranoside (**1**) was prepared by conventional acetylation of *p*-nitrophenyl α-L-arabinofuranoside (**2**), using acetanhydride in pyridine. *p*-Nitrophenyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside (**3**) (Scheme 1) was obtained through hydrolysis of primary acetyl group of **1** by lipase from *Candida cylindracea* (CCL) or lipase from *Candida rugosa* (LAY). In both cases, the starting triacetate **1** was completely consumed after 4 h and product **3** was isolated as a single crystalline compound in 90% yield after purification. A similar selective removal of the primary acyl in esterified monosaccharides was obtained under the action of CCL or LAY.[11,16] No acetyl group migration[1] in product **3** was observed in used aqueous media, only a very slow spontaneous deacetylation took place during the reaction.

2.2. Preparation of *p*-nitrophenyl 2,5- and 3,5-di-*O*-acetyl-α-L-arabinofuranoside (4 and 5)

Of several enzymes, tested for preparation of the two remaining di-*O*-acetates, the lipase from *Pseudomonas cepacia* (LPS-30) was found to acetylate smoothly *p*-nitrophenyl α-L-arabinofuranoside (**2**). A mixture of di-*O*-acetates **4** and **5** was formed in high yields in different organic solvents (Scheme 2 and Table 1). Solvents possessing different polarity were examined. The reactions were performed at 40 °C and were stopped after almost all *p*-nitrophenyl 5-*O*-acetyl-α-L-arabinofuranoside (**6**) was converted to diacetates. The course of acetylation of furanoside **2** was monitored by HPLC. Analysis of the results presented in Table 1 suggests that activity of LPS-30 depends on polarity of the solvents used. The reaction proceeded more rapidly in less polar solvents. The time of di-*O*-acetylation of **2** catalysed by LPS-30 in diisopropyl ether was comparable to the time of the conventional chemical acetylation. The comparison of the reaction times in individual organic solvents (Table 1) with those of LPS-30-catalysed acetylation of *p*-nitrophenyl xylopyranoside under appropriate conditions[1] is in favour of arabinofuranoside **2**. Solvent polarity showed little effect on the final proportion of the diacetates. In most solvents, a mixture of 2,5- and 3,5-diacetate (**4** and **5**) was produced in a ratio about 1:3, regardless of their hydrophobicity (Table 1). This fact is in contradiction with our previous observation that the regioselectivity of the di-*O*-acetylation of *p*-nitrophenyl xylopyranoside[1] was affected by polarity of the used organic solvents. Small amounts of the triacetate **1** and monoacetate **6** were also formed. Based on these results, one may conclude that arabinofuranoside **2** has a weaker interaction with the enzyme active site than pyranoside derivatives studied previously.[1,32,33] We did not find conditions to produce solely 5-*O*-acetate **6** upon catalysis by LPS-30.



Scheme 1. Reagents and conditions: (a) CCL or LAY, 10% DMF in 0.1 M phosphate buffer (pH 7), 37 °C, 4 h.

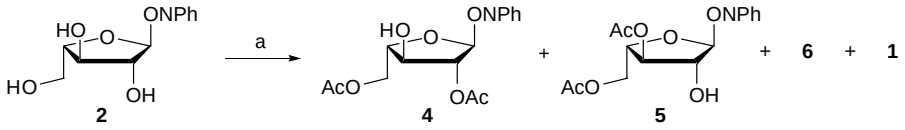
2.3. Preparation of *p*-nitrophenyl 5-*O*-acetyl-α-L-arabinofuranoside (6)

We also focused on preparation of monoacetates of *p*-nitrophenyl α-L-arabinofuranoside. The problem to terminate the acetylation of **2** by LPS-30 at the stage of the monoacetate **6** was overcome using the porcine pancreas lipase (PPL) frequently applied for the acylation of primary hydroxyl group of monosaccharides.[11,16] Using vinyl acetate as an acetate donor, PPL in THF catalysed selective synthesis of 5-monoacetate **6** from **2** in 95% yield (Scheme 3) and satisfactory reaction time.

2.4. Preparation of *p*-nitrophenyl 2- and 3-*O*-acetyl-α-L-arabinofuranoside (7 and 8)

Among commercially available lipases and proteases, we did not find any preparation able to catalyse acetylation of unprotected monosaccharides selectively to position 2-OH or 3-OH. That is why two monoacetates **7** and **8** were obtained from 2,5- and 3,5-di-*O*-acetates by hydrolysis of the primary OAc moiety by CCL. The 2-*O*-acetate **7** was produced from 2,5-diacetate **4** in 80% yield (Scheme 4). Slightly acidic pH 6 was used to diminish spontaneous hydrolysis of acetates. From Fig. 1A and Table 2 (entry 4) it is evident that a small amount of 5-*O*-acetate **6** was formed beside the desired product **7**. The 5-*O*-acetate **6** was then rapidly converted to **2** by CCL.

The trend to hydrolyse the secondary OAc group beside the primary one was stronger during treatment of 3,5-*O*-diacetate **5** by CCL. The reaction led to formation of several products—monoacetates **6**, **8** and completely deacetylated **2**. The desired 3-*O*-acetate **8** was obtained in 50–55% yield. These results are explained by parallel deacetylation of 5-OAc and 3-OAc of 3,5-di-*O*-acetyl-α-L-arabinofuranoside **5** (Scheme 5). The primary 5-OAc group was hydrolysed away faster than the secondary 3-OAc group of **8**, which seems to be a kinetically preferred product (Fig. 1B). Similar product composition was observed at different pH (Table 2, entries 5 and 6). Decrease in the temperature did not reduce the formation of by-products (Table 2, entry 7).

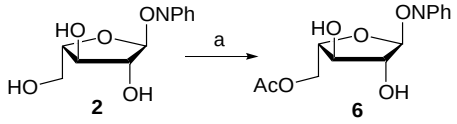


Scheme 2. Reagents: (a) LPS, vinyl acetate and organic solvent (Table 1).

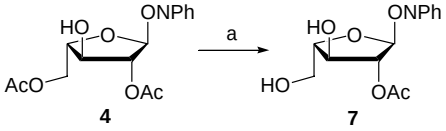
Table 1. Results of acetylation of **2** by LPS-30 in organic solvents at 40 °C

Entry	Organic solvent	Log P_{ow}[a]	Reaction time (h)	Conversion (**4** + **5**) (%)	Ratio (**4**:**5**)	**6** (%)	**1** (%)
1	Cyclohexane	3.40	76	64	1:1.1	0	36
2	Toluene	2.69	75.5	91	1:3.6	0	9
3	Diisopropyl ether	1.90	23.5	97	1:2.7	0	3
4	Methyl isobutyl ketone	1.38	77	96	1:2.3	2	2
5	Ethyl acetate	0.73	147	93	1:3.1	1	6
6	Vinyl acetate	0.73	51	96	1:3.6	0	3
7	*tert*-Butanol	0.40	146	97	1:3.0	1	2
8	Acetonitrile	−0.33	146.5	93	1:3.2	1	6

[a] Octanol/water partition coefficient.



Scheme 3. Reagents and conditions: (a) PPL, vinylacetate, THF, 15 h, 95%.



Scheme 4. Reagents and conditions: (a) CCL, 10% DMF in 0.1 M phosphate buffer (pH 6), 37 °C, 7 h, 80%.

The fact, that during hydrolysis of the primary acetyl group of diacetates **4** or **5** the secondary OH groups (3-OH more than 2-OH) were also partially deacetylated, motivated us to treat pure 2,3-diacetate **3** with CCL under similar conditions like **4** and **5**. Interestingly, diacetate **3** exhibited high stability under these conditions (Table 2, entry 3). No loss of **3** was recorded after a 45.5 h treatment by CCL at 37 °C.

Next, to examine the effect of the acetyl migration or spontaneous deacetylation on the results previously observed, **3** was incubated several days in phosphate buffer (pH 6) at 40 °C according to our previous studies with partially acetylated *p*-nitrophenyl xylopyranoside.[1]

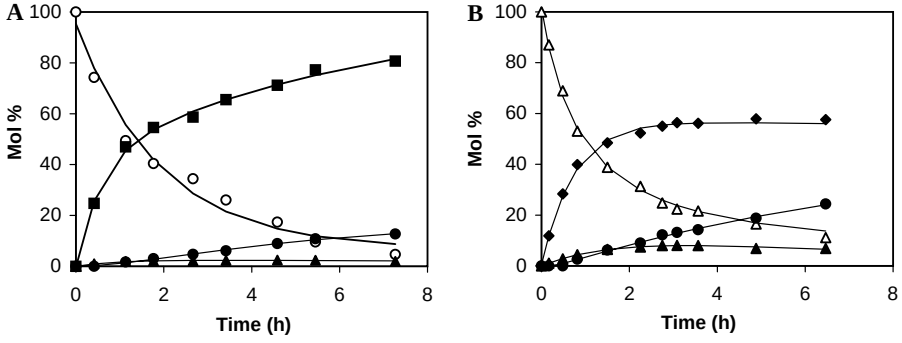
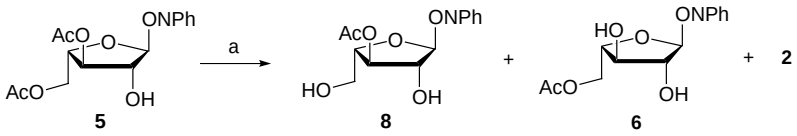


Figure 1. Time course of conversion of di-*O*-acetates **4** (A), **5** (B) into compounds **6**, **7**, **8** and **2** during incubation with CCL in phosphate buffer (pH 6) at 37 °C. Symbols: (○), **4**; (△), **5**; (▲), **6**; (■), **7**; (♦), **8**; (●), **2**.

Table 2. HPLC results of enzymatic hydrolysis of acetates **1**, **4** and **5** by LCC, resp. LAY at 37 °C in 0.033 M solution of DMF/0.1 M phosphate buffer, 1:9

Entry	SC[a]	pH	Lipase	Time[b] (h)	**1**	**3**	**4**	**5**	**6**	**7**	**8**	**2**
1	**1**	6	CCL	5.8	4.7	94.4				0.9		0
2	**1**	7	CCL	3.7	4.2	95.0				0.8		0
3	**3**	6	CCL	45.5		100						
4	**4**	6	CCL	7.3			4.7		1.9	80.7		12.7
5	**5**	6	CCL	4.6				17.9	8.3		55.5	17.3
6	**5**	6	LAY	4.9				16.5	6.8		57.9	18.8
7	**5**	7	CCL	6.2[c]				22.8	8.0		52.6	16.5

[a] Starting compound.
[b] Time of maximal concentration of the desired products in reaction mixtures.
[c] Reaction at 30 °C.



Scheme 5. Reagents and conditions: (a) CCL or LAY, 10% DMF in 0.1 M phosphate buffer (pH 6) (Table 2, entries 5–7).

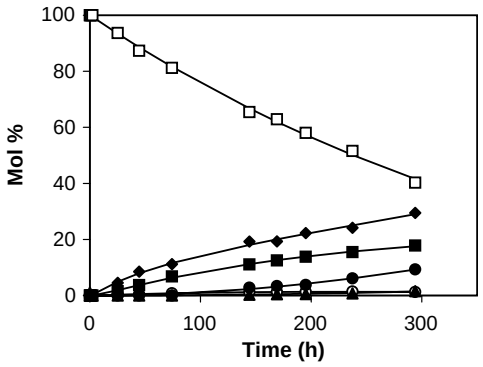


Figure 2. Time course of conversion of acetate **3** during incubation in phosphate buffer (pH 6) at 40 °C. Symbols: (□), **3**; (○), **4**; (△), **5**; (▲), **6**; (■), **7**; (♦), **8**; (●), **2**.

HPLC analysis showed (Fig. 2) that the acetyl migration in **3** did not proceed or proceeded extremely slowly under these conditions. Only spontaneous deacetylation was monitored. The deacetylation from 2-OAc group is more rapid due to a higher reactivity of O-2 resulting from the inductive effect of the anomeric centre.

3. Conclusion

The regioselectivity of acetylation and deacetylation of derivatives of *p*-nitrophenyl α-L-arabinofuranoside using lipases was studied. All six possible partially acetylated derivatives of *p*-nitrophenyl α-L-arabinofuranoside have been prepared. The 2- and 3-*O*-acetates of *p*-nitrophenyl α-L-arabinofuranoside were prepared in two steps. The first step was the enzymatic di-*O*-acetylation and the second one was enzymatic hydrolysis of the primary acetyl group. The prepared di-*O*-acetates have been used for the syntheses of carbohydrate ferulates[30] through enzymatic protection, feruloylation and chemical deacetylation. The monoacetates will be used to study substrate specificity and substrate structure requirements of α-L-arabinofuranosidases and carbohydrate esterases. These prepared partially acetylated arabinofuranosides can also serve as building blocks for the synthesis of L-arabinooligosaccharides. Neither mononor di-*O*-acetates of *p*-nitrophenyl α-L-arabinofuranoside underwent significant spontaneous acetyl group migration in aqueous media.

4. Experimental

4.1. General

Lipase from *C. rugosa* (LAY-30) and lipase from *Burkholderia cepacia*, syn. *P. cepacia* (LPS-30) were gifts from Amano (Japan). Lipase from *C. cylindracea*, syn. *C. rugosa* (CCL) was a gift from Lyven (France). The porcine pancreas lipase (PPL) was purchased from Sigma. Solvents were distilled from the appropriate drying agents before use. The *p*-nitrophenyl α-L-arabinofuranoside **2** was purchased from Sigma or prepared by glycosylation of 1,2,3,5-tetra-*O*-acetyl-α,β-L-arabinofuranose[34] with *p*-nitrophenol using $SnCl_4$ in CH_2Cl_2 followed by treatment with 0.09 mM MeONa/MeOH in 60% yield. All reactions were monitored on TLC plates (Silicagel 60, 0.25 mm, E. Merck, Darmstadt, Germany). The compounds were detected by charring the plates with 1% orcinol in 10% (v/v) ethanolic solution of H_2SO_4 at ca. 200 °C. Column chromatography was performed on Silica gel (0.035–0.070 mm, pore diameter ca. 6 nm, Acrōs Organics) in various solvent systems. Melting points were determined with a Kofler hot-stage and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20 °C. HPLC analysis was carried out on a chromatograph equipped with a silica gel column (Biospher Si 100 5 μm). The elution of acetates of *p*-nitrophenyl α-L-arabinofuranoside was monitored with a calibrated UV detector at 305 nm. The elution times in hexane/ethyl acetate, 3:2 and a rate of 1.0 mL/min were 2.0 min for **1**, 2.5 min for **5**, 3.1 min for **4**, 3.6 min for **3**, 4.7 min for **8**, 9.1 min for **6**, 12.33 min for **7** and 30.5 min for **2**; alternatively in hexane/ethyl acetate, 1:1 and a rate of 1.5 mL/min, they were 1.4 min for **1**, 1.6 min for **5**, 1.7 min for **4**, 1.8 min for **3**, 2.1 min for **8**, 2.8 min for **6**, 3.3 min for **7** and 12.4 min for **2**. The quantitation was done by integration of the peak areas. ^{1}H NMR spectra were recorded at 300 MHz with Bruker AM 300 (Me_4Si as internal standard). Homonuclear correlation two-dimensional technique COSY-45 was used for complete assignment of protons. ^{13}C NMR spectra were recorded in $CDCl_3$ at 75 MHz and shifts are referenced to internal $CDCl_3$. Microanalyses were performed with a Fisons EA 1108 analyser.

4.2. *p*-Nitrophenyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside (3)

p-Nitrophenyl 2,3,5-tri-*O*-acetyl-α-L-arabinofuranoside (**1**) (0.4 g, 1 mmol) was dissolved in DMF (3 mL) and then 0.1 M phosphate buffer pH 7 (27 mL) was added followed by addition of lipase CC (0.8 g). The reaction mixture was shaken at 37 °C and 200 rpm. After 220 min, the HPLC monitoring (hexane/ethyl acetate, 3:2, rate 1 mL/min) showed transformation of **1** to 95% of **3** and 0.8% of **7**. The reaction was stopped by extraction of saccharidic material from reaction mixture to ethyl acetate. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The product **3** was separated from the starting compound and traces of

7 by column chromatography (toluene/ethyl acetate, 2:1) to give **3** as a white solid (0.32 g, 90%). Mp = 77–79 °C (isopropyl alcohol/diisopropyl ether); $[\alpha]_D^{20}$ −141.0 (*c* 1.0, $CHCl_3$); [lit. [35] 77–79 °C (diisopropyl ether); $[\alpha]_D^{20}$ −142 (*c* 1.2, $CHCl_3$)]. ^{1}H NMR (300 MHz, $CDCl_3$) δ d 1.63 (br s, 1H, OH), 2.15 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 3.84 (dd, 1H, $J_{4,5}$ 4.0 and $J_{5,5'}$ 12.3 Hz, H-5), 3.92 (dd, 1H, $J_{4,5'}$ 3.4 and $J_{5,5'}$ 12.3 Hz, H-5′), 4.25 (dt, 1H, H-4), 5.20 (dd, 1H, $J_{3,4}$ 5.2 and $J_{2,3}$ 1.8 Hz, H-3), 5.43 (d, $J_{1,2}$ 0 and $J_{2,3}$ 1.8 Hz, H-2), 5.9 (d, 1H, H-1), 7.15 (d, 2H, *J* 9.2 Hz, H-2′, H-6′), 8.21 (d, 2H, *J* 9.2 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ d 20.8 ($2\times CO\mathit{C}H_3$), 61.7 (C-5), 76.6 (C-3), 81.6 (C-2), 84.6 (C-4), 103.7 (C-1), 2×116.6, 2×125.8, 142.8, 160.8 ($6\times$C-Ar), 169.7 ($\mathit{C}OCH_3$), 170.5 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{15}H_{17}NO_9$: C, 50.71; H, 4.82; N, 3.94. Found: C, 50.42; H, 5.17; N, 3.69.

4.3. HPLC test for the acetyl migration

Di-*O*-acetate **3** (0.002 mmol) was dissolved in DMSO (80 μL) and mixed with 0.1 M sodium phosphate buffer (1920 μL) of pH 6 preheated to 40 °C. The reaction mixtures were incubated at 40 °C for several days. The aliquots (50 μL) were withdrawn at time intervals and directly injected to the HPLC system through a 20 μL loop using a silica gel column eluted with hexane/ethyl acetate, 3:2, rate 1 mL/min. The graphical illustration of the monitored process is shown in Figure 2.

4.4. Monitoring of di-*O*-acetylation of *p*-nitrophenyl α-L-arabinofuranoside

p-Nitrophenyl α-L-arabinofuranoside (**2**) (27.1 mg, 0.1 mmol) was dissolved in a selected organic solvent (954 μL) and then molecular sieve (4 Å, 250 mg), vinyl acetate (46 μL, 5 equiv) and 270 mg LPS were added. The reaction mixtures were shaken at 40 °C at 200 rpm. Aliquots were withdrawn in different time intervals and filtered (Durapore filter, 0.45 μm). The filtered solutions were analysed by HPLC on a silica gel column in hexane/ethyl acetate, 1:1, at an elution rate of 1.5 mL/min. The ratios of compounds, calculated from the integrated peak areas, are shown in Table 1.

4.5. *p*-Nitrophenyl 2,5- and 3,5-di-*O*-acetyl-α-L-arabinofuranoside (4 and 5)

p-Nitrophenyl α-L-arabinofuranoside (**2**) (1 g, 3.7 mmol) was suspended in diisopropyl ether (300 mL). Molecular sieves (4 Å, 5 g), vinyl acetate (3.4 mL, 37 mmol, 10 equiv) and lipase PS (9 g) were added. The reaction mixture was shaken at 37 °C and 200 rpm for 30 h. The reaction was stopped by filtration off the lipase, which was then repeatedly used in four subsequent reaction cycles. The filtrate was concentrated and the residue was fractionated by column chromatography (toluene/ethyl acetate, 5:1 → 2:1) to give first **1** as an oil (0.06 g, 7%), then **5** as a white solid (0.48 g, 61%). Mp = 67–68 °C (diisopropyl ether/cyclohexane). $[\alpha]_D^{20}$ −200.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ d 2.12 (s, 3H, $COCH_3$), 2.18 (s, 3H, $COCH_3$), 3.21 (br s, 1H, OH), 4.29 (dd, 1H, $J_{4,5}$ 4.7 and $J_{5,5'}$ 12.3 Hz, H-5), 4.41 (dd, 1H, $J_{4,5'}$ 3.1 and $J_{5,5'}$ 12.5 Hz, H-5′), 4.45 (m, 1H, H-4), 4.48 (br d, 1H, H-2), 4.80 (dd, 1H, $J_{2,3}$ 2.7 and $J_{3,4}$ 6.3 Hz, H-3), 5.76 (s, 1H, H-1), 7.12 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 8.21 (d, 2H, *J* 9.3 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz $CDCl_3$) δ d 20.7 ($2\times CO\mathit{C}H_3$), 62.7 (C-5), 80.5, 80.8, 81.2 (C-2, C-3, C-4), 105.8 (C-1), 2×116.4, 2×125.8, 142.6, 161.1 ($6\times$C-Ar), 170.5 ($\mathit{C}OCH_3$), 171.8 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{15}H_{17}NO_9$: C, 50.71; H, 4.82, N, 3.94. Found: C, 50.71; H, 5.00; N, 3.98. Next eluted was oily **4** (0.33 g 25%). $[\alpha]_D^{20}$ −156.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ d 2.11 (s, 3H, $COCH_3$), 2.18 (s, 3H, $COCH_3$), 3.32 (br s, 1H, OH), 4.12 (dd, 1H, $J_{2,3}$ 3.4 and $J_{3,4'}$ 6.4 Hz, H-3), 4.27 (dd, 1H, $J_{4,5}$ 6.3 and $J_{5,5'}$ 12.9 Hz, H-5), 4.36 (m, 1H, H-4), 4.38 (dd, 1H, $J_{4,5'}$ 3.2 and $J_{5,5'}$ 13.0 Hz, H-5′), 5.18 (dd, $J_{1,2}$ 1.1 and $J_{2,3}$ 3.4 Hz, H-2), 5.81 (d, $J_{1,2}$ 1.1 Hz, H-1), 7.14 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 8.21 (d, 2H, *J* 9.3 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ d 20.7 ($2\times CO\mathit{C}H_3$), 62.8 (C-5), 76.9 (C-3), 82.1 (C-4), 86.1 (C-2), 103.7 (C-1), 2×116.5, 2×125.7, 142.7, 160.9 ($6\times$C-Ar), 170.8 ($\mathit{C}OCH_3$), 171.4 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{15}H_{17}NO_9$: C, 50.71; H, 4.82, N, 3.94. Found: C, 50.57; H, 5.07; N, 3.53.

4.6. *p*-Nitrophenyl 5-*O*-acetyl-α-L-arabinofuranoside (6)

p-Nitrophenyl α-L-arabinofuranoside **2** (0.27 g, 1 mmol) was dissolved in THF (25 mL) and vinyl acetate (1.4 mL, 15 mmol) followed by PPL (1 g) was added. The reaction mixture was shaken at 40 °C and 200 rpm for 15 h. The reaction was stopped by filtering off the lipase. The filtrate was concentrated and the residue was purified by column chromatography (toluene/ethyl acetate, 1:2) to give only monoacetate **6** (0.30 g, 95%) as a colourless oil. $[\alpha]_D^{20}$ −133 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ d 2.12 (s, 3H, $COCH_3$), 2.81 (br s, 2H, OH), 4.10 (dd, 1H, $J_{2,3}$ 3.8 and $J_{3,4}$ 5.1 Hz, H-3), 4.29–4.34 (m, 3H, H-4, H-5, H-5′), 4.49 (dd, 1H, $J_{1,2'}$ 1.6 and $J_{2,3'}$ 3.7 Hz, H-2), 5.71 (d, $J_{1,2}$ 1.5 Hz, H-1), 7.12 (d, 2H, *J* 9.2 Hz, H-2′, H-6′), 8.18 (d, 2H, *J* 9.2 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ d 20.8 ($CO\mathit{C}H_3$), 63.4 (C-5), 77.8 (C-3), 81.4 (C-2), 83.6 (C-4), 106.0 (C-1), 2×116.5, 2×125.8, 142.7, 161.7 (C-Ar), 170.8 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{13}H_{15}NO_8$: C, 49.84; H, 4.83, N, 4.47. Found: C, 50.06; H, 5.01; N, 4.05.

4.7. *p*-Nitrophenyl 2-*O*-acetyl-α-L-arabinofuranoside (7)

2,5-Diacetate **4** (0.18 g, 0.5 mmol) was dissolved in DMF (1.5 mL), then 0.1 M phosphate buffer, pH 6 (13.5 mL), was added followed by addition of CCL (0.1 g). The reaction mixture was shaken at 37 °C and 200 rpm for 7 h. Then ethyl acetate was added to the reaction mixture and products were extracted from the aqueous mixture. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The 2-acetate **7** was separated from **2** and traces of the starting compound and **6** by column chromatography (toluene/ethyl acetate, 1:1) to give **7** as a colourless oil (0.11 g, 76%). $[\alpha]_D^{20}$ −160 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$)

δ d 2.18 (s, 3H, $COCH_3$), 3.80 (dd, 1H, $J_{4,5}$ 1.6 and $J_{5,5'}$ 12.8 Hz, H-5), 3.96 (dd, 1H, $J_{4,5'}$ 2.0 and $J_{5,5'}$ 12.2 Hz, H-5′), 4.26 (m, 2H, H-3, H-4), 5.16 (br s, 1H, H-2), 5.84 (d, $J_{1,2}$ 1.2 Hz, H-1), 7.14 (d, 2H, J 9.3 Hz, H-2′, H-6′), 8.22 (d, 2H, J 9.3 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ d 20.8 ($CO\mathit{C}H_3$), 61.1 (C-5), 76.1 (C-3), 84.3 (C-4), 87.1 (C-2), 103.7 (C-1), 2×116.5, 2×125.8, 142.7, 161.0 ($6\times$C-Ar), 171.7 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{13}H_{15}NO_8$: C, 49.84; H, 4.83; N, 4.47. Found: C, 50.11; H, 5.07; N, 4.09.

4.8. *p*-Nitrophenyl 3-*O*-acetyl-α-L-arabinofuranoside (8)

3,5-Diacetate **5** (0.36 g, 1 mmol) was dissolved in DMF (3 mL) and then 0.1 M phosphate buffer, pH 6 (27 mL) and lipase CC (0.8 g) were added. The reaction mixture was shaken at 37 °C and 200 rpm for 4.5 h when concentration of **8** reached the maximum according to HPLC (hexane/ethyl acetate, 1:1, rate 1.5 mL/min). The reaction was stopped by extraction of the reaction material to ethyl acetate. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The product **8** was purified from starting compound **5**, formed **2** and traces of **6** by column chromatography (toluene/ethyl acetate, 2:1) to give **8** as a white solid (0.16 g, 51%). Mp = 107–108 °C (diisopropyl ether); $[\alpha]_D^{20}$ −186 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ d 2.17 (s, 3H, $COCH_3$), 3.91 (dd, 1H, $J_{4,5}$ 2.0 and $J_{5,5'}$ 11.9 Hz, H-5), 3.96 (dd, 1H, $J_{4,5'}$ 2.3 and $J_{5,5'}$ 11.9 Hz, H-5′), 4.33 (m, 1H, H-4), 4.46 (br s, 1H, H-2), 5.01 (dd, 1H, $J_{2,3}$ 1.3 and $J_{3,4}$ 4.0 Hz, H-3), 5.79 (s, 1H, H-1), 7.12 (d, 2H, J 9.2 Hz, H-2′, H-6′), 8.21 (d, 2H, J 9.2 Hz, H-3′, H-5′). ^{13}C NMR (75 MHz, $CDCl_3$) δ d 20.8 ($CO\mathit{C}H_3$), 61.5 (C-5), 79.6, 79.8 (C-2, C-3), 84.7 (C-4), 106.2 (C-1), 2×116.3, 2×125.8, 142.4, 161.2 ($6\times$C-Ar), 171.4 ($\mathit{C}OCH_3$). Anal. Calcd for $C_{13}H_{15}NO_8$: C, 49.84; H, 4.83; N, 4.47. Found: C, 50.01; H, 4.96; N, 4.19.

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