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Deoxy and deoxyfluoro analogues of acetylated methyl β-D-xylopyranoside—substrates for acetylxylan esterases

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Abstract—Four modified substrates for acetylxylan esterases, 2-deoxy, 3-deoxy, 2-deoxy-2-fluoro, and 3-deoxy-3-fluoro derivatives of di-*O*-acetylated methyl β-D-xylopyranoside were synthesized via 2,3-anhydropentopyranoside precursors. Methyl 2,3-anhydro-4-*O*-benzyl-β-D-ribopyranoside was transformed into methyl 2,3-anhydro-4-*O*-benzyl-β-D-lyxopyranoside in three steps. The epoxide ring opening of 2,3-anhydropentopyranosides was accomplished either by hydride reduction or hydrofluorination. Methyl β-D-xylopyranoside 2,3,4-tri-*O*-, 2,4-di-*O*-, and 3,4-di-*O*-acetates, and the prepared diacetate analogues were tested as substrates of acetylxylan esterases from *Schizophyllum commune* and *Trichoderma reesei*. Measurement of their rate of deacetylation pointed to unique structural requirements of the enzymes for the substrates. The enzymes differed particularly in the requirement for the *trans* vicinal hydroxy group in the deacetylation at C-2 and C-3 and in the tolerance to the presence of *trans* vicinal acetyl groups esterifying the OH group at C-2 and C-3.

Keywords: 2,3-Anhydropyranosides; Deoxy and deoxyfluoro sugars; Acetylxylan esterase specificity

1. Introduction

Microbial acetylxylan esterases (EC 3.1.1.72, AcXEs) represent a group of carbohydrate esterases with great potential in biotechnology and carbohydrate chemistry. They deacetylate partially acetylated 4-*O*-methyl-D-glucuronoxylan, the main hardwood hemicellulose, or its fragments generated upon the action of endo-β-(1→4)-xylanases. Different enzymes with AcXE activity have recently been purified from various microorganisms.[1,2] The three-dimensional structure of AcXEs from *Penicillium purpurogenum*[3] and *Trichoderma reesei*[4] has been established. Both enzymes belong to CE family 5 and are typical serine esterases. Recent studies on the mode of action of AcXEs from *T. reesei*[5] *Schizophyllum commune*[6] and *Streptomyces lividans*[7] on fully or partially acetylated methyl β-D-xylopyranoside (D-Xyl*p*βOMe) revealed an interesting regioselectivity of substrate deacetylation. The enzymes, in general, deacetylated positions 2 and 3, which are compatible with their function in biodegradation of hemicellulose. A unique behavior was observed with AcXE from *S. lividans*[7] belonging to the carbohydrate esterase (CE) family 4.[8] This esterase deacetylated very slowly 2,3-di-*O*-Ac-D-Xyl*p*βOMe and 2,3,4-tri-*O*-Ac-D-Xyl*p*βOMe. The first deacetylation step was, however, immediately followed by a second one, yielding 4-*O*-Ac-D-Xyl*p*βOMe as the main reaction product. The double deacetylation became better understood after finding that 2,4-di-*O*-Ac and 3,4-di-*O*-Ac-D-Xyl*p*βOMe were deacetylated by several orders of magnitude faster as compared to 2,3,4-tri-*O*-Ac-D-Xyl*p*βOMe and 2,3-di-*O*-Ac-D-Xyl*p*βOMe.[7] Since it was difficult to imagine that the enzyme has an equal ability to deacetylate positions 2 and 3, we proposed that release of the acetyl group in these two positions, in case the neighboring hydroxyl at C-3 or C-2 is nonacetylated, involves a common 2,3-*ortho* ester intermediate. Such structure is believed to

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take part in the acetyl group migration along the pyranoid ring of aldoses.[9] A similar *ortho* acid intermediate was proposed in partial reactions catalyzed by peptidyltransferases (aminoacyl-*t*RNA synthases) as a result of an easy aminoacyl group migration between *cis*-related 2- and 3-OH groups of ribose in the terminal nucleotide.[10]

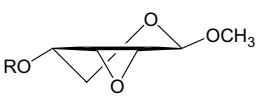
Verification of the hypothesis that *ortho* ester intermediates or acetyl migration[6,7] or reorientation of a residue[4] can take part in the mechanism of deacetylation of AcXE from *S. lividans* requires substrate analogues of acetylated β-D-Xyl*p*βOMe in which the formation of an hypothetical 2,3-*ortho* ester or migration of acetyl group is ruled out. Such analogues are acetylated derivatives of 2-deoxy-, 3-deoxy-, 2-deoxy-2-fluoro- and 3-deoxy-3-fluoro-D-Xyl*p*βOMe. A preliminary report on the synthesis of these analogues was already published.[11] The analogues have also been used in a study of the mode of action of AcXE from *S. lividans*, a carbohydrate esterase belonging to family 4.[12] Replacement of the OH-group at C-2 or C-3 by hydrogen or fluorine resulted in a net decrease of the rate of deacetylation at the neighboring position, pointing an essential role of the vicinal OH-group in the mechanism of deacetylation by the *S. lividans* AcXE.[12]

In this report, we present the full description of the preparation and characterization of di-O-acetylated 2-deoxy, 3-deoxy, 2-deoxy-2-fluoro and 3-deoxy-3-fluoro derivatives of D-Xyl*p*βOMe, and their use in studies of the mode of action of two AcXEs belonging to the carbohydrate esterase families 1 and 5.[8] The response of each enzyme to the replacement of the vicinal OH-group in D-Xyl*p*βOMe diacetates was compared with the behavior of *S. lividans* AcXE toward the same derivatives.[12] Each AcXE was found to be unique in terms of role of the vicinal OH-group in the deacetylation mechanism.

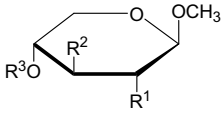
2. Results and discussion

2.1. Synthesis of deoxy and deoxyfluoro analogues of D-Xyl*p*βOMe diacetates (Chart 1)

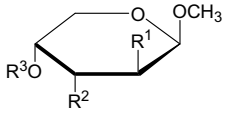
Methyl 2,3-anhydro-β-D-ribopyranoside (**1**) was prepared from D-arabinose.[13] Benzylation of **1** yielded methyl 2,3-anhydro-4-*O*-benzyl-β-D-ribopyranoside (**2**),[14] the precursor for the preparation of methyl 2,3-anhydro-4-*O*-benzyl-β-D-lyxopyranoside (**5**). The decisive step involved opening of the epoxide ring of **2** using 10 equiv of sodium acetate in acetic acid at 130 °C, which gave as major compound methyl 3-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranoside (**3**). The reaction was modified according to Lugemwa and Denison[15] It proceeded with high regioselectivity since only the 3-*O*-acetate **3** and the 2-*O*-acetate **4** of methyl 4-*O*-benzyl-β-D-xylopyranoside were formed (91% overall yield). Probably due to the *trans*-acetyl migration[9] from O-3 to O-2 due to both the presence of base and the high reaction temperature, only 66% of the 3-*O*-acetate **3** was isolated. In order to increase the yield of **3**, we were able to transform almost quantitatively the undesired 2-*O*-acetate **4** to the 3-*O*-acetate **3** chemoenzymatically. The C-3 hydroxyl group of methyl 4-*O*-benzyl-β-D-xylopyranoside obtained by Zemplén deacetylation of **4** was acetylated regioselectively with vinyl acetate in the presence of a catalytic amount of lipase PS-30 (Amano).



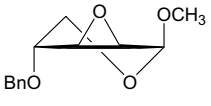
	R
1	H
2	Bn



	R^1	R^2	R^3
3	OH	OAc	Bn
4	OAc	OH	Bn
6	OH	H	H
7	OH	F	H
9	H	OH	Bn
11	F	OH	Bn
14	H	OH	H
15	F	OH	H
16	OAc	H	Ac
17	OAc	F	Ac
18	H	OAc	Ac
19	F	OAc	Ac



	R^1	R^2	R^3
8	F	OH	H
10	OH	H	Bn
12	OH	F	Bn



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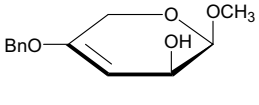

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Chart 1.

2,3-Anhydro-4-*O*-benzyl-β-D-lyxopyranoside **5** was obtained in 87% yield after tosylation of **3** and alkaline treatment of the resulting crude tosylate with sodium methoxide in methanol. The 4-*O*-benzyl group of **5** avoids the possibility of equilibrium between the first produced D-*lyxo*-2,3-epoxide and the D-*arabino*-3,4-epoxide formed by epoxide-ring migration. The ^{1}H NMR spectra established the structure of **5** by the shifts of H-2 and H-3 (δ 3.33 and 3.37 ppm) and by the small coupling constants $J_{1,2}, J_{2,3}$ and $J_{3,4}$ (2.9, 3,7 and 1.9 Hz), typical for 2,3-anhydropentopyranosides.

The prepared 2,3-anhydropentopyranosides **1** and **5** were converted to deoxy and deoxyfluoro analogues of methyl β-D-xylopyranoside **6**, **7**, **9** and **11** by regioselective epoxide ring opening of **1** and **5**, either by reduction or hydrofluorination. The known D-*ribo*-2,3-epoxides **1** or **2** could be regioselectively transformed to 3-substituted xylopyranosides by preferential nucleophilic attack at C-3 as indicated in previous studies.[13b,14,16] There is however no mention about the existence of side products. As an extension of these results, we observed that this reaction proceeds regioselectively also with small nucleophiles like H^- and F^- (Table 1). We paid also attention to the detection of possible side products. Reduction of epoxide **1** by treatment with $LiAlH_4$ in THF at room temperature gave exclusively the corresponding 3-deoxy-β-D-*erythro*-pentopyranoside **6** in 74% yield after purification from the starting compound **1**. The minor methyl 2-deoxy-2-fluoro-β-D-arabinopyranoside (**8**) was isolated in 6% yield together with 70% of the desired 3-deoxy-3-fluoro-β-D-xylopyranoside (**7**) when **1** was hydrofluorinated with $Bu_4N^+H_2F_3^-$ and KHF_2 at 130 °C under solid-liquid PTC[17] (Table 1).

The possibility of transformation of the newly prepared methyl 2,3-anhydro-4-*O*-benzyl-β-D-lyxopyranoside (**5**) into the corresponding C-2 modified β-D-xylopyranosides was also studied. Reduction under the same conditions as used for the D-*ribo*-2,3-epoxide **1** afforded D-*xylo* and D-*arabino* products in the proportion 1.8:1 (Table 1). After column chromatography on silica gel, methyl 4-*O*-benzyl-2-deoxy-β-D-*threo*-pentopyranoside (**9**) and methyl 4-*O*-benzyl-3-deoxy-β-D-*threo*-pentopyranoside (**10**) were obtained in 53% and 29% yield, respectively. However, when the epoxide **5** was hydrofluorinated with $Bu_4N^+H_2F_3^-/KHF_2$ under the SL–PTC conditions, the unsaturated product **13** was isolated from the reaction mixture in 56% yield. Additionally, the 3-deoxy-3-fluoro-β-D-arabinopyranoside derivative **12** was obtained in 15% yield whereas the desired 2-deoxy-2-fluoro-β-D-xylopyranoside derivative **11** was isolated in 6% yield only by column chromatography. Alternative treatment of **5** by KHF_2 in ethane-1,2-diol at 180 °C resulted in an absence of regioselectivity (Table 1) due to the high temperature, but satisfactorily afforded 4-*O*-benzyl-2-deoxy-2-fluoro-β-D-xylopyranoside (**11**) (36%), which is inaccessible by the DAST method due to the occurrence of 1,2-aglycon migration.[18]

The drop in regioselectivity and yields of 2-substituted xylopyranosides from epoxide **5** through *trans*-diaxial ring opening according to the Fürst–Plattner rule may result from electrostatic and steric repulsions of the incoming nucleophile as well as adoption of a $^5H_0$ transition state conformation during the reaction due to the anomeric effect and electrostatic repulsion of the lone-pair electrons of the pyranoid ring and epoxide ring oxygens.[19] The vicinal coupling constants for H-4, H-5a, and H-5b of **5** measured in $CDCl_3$ at room temperature are small ($J_{4,5a}$ 3.1 Hz and $J_{4,5b}$ 2.1 Hz) and we also observed a long-range W-coupling between the equatorial H-3 and H-5 ($^4J_{3,5a}$ 1.6 Hz).

Catalytic hydrogenation (10% palladium on charcoal) removed the benzyl group from **9** and **11** to yield the glycosides **14** and **15**. Finally, the resulting deoxy- and deoxyfluoro-β-D-xylopyranosides were fully *O*-acetyl-ated to afford the target modified AcXE substrates **16**–**19**.

The structures of all methyl β-D-xylopyranoside derivatives were proven by ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy. Confirmation of the position of reduction and fluorination in the modified pentopyranosides was based on two-dimensional H–H COSY and HMQC techniques and analysis of geminal, vicinal and long-range H–H and F–H coupling constants in one-dimensional ^{1}H NMR spectra, respectively. For example, the geminal coupling constants $^2J_{F,H}$ ranged from 52.0 to 49.5 Hz for xylopyranosides **7**, **11**, **15**, **17**, and **19** and for arabinopyranosides **8** and **12** values of $^2J_{F,H}$ were 48.5 and 48.4 Hz, respectively. The proton broad band decoupled ^{13}C NMR spectra of **16**–**19** showed that the

Table 1. Epoxide ring opening of **1** and **5**

Starting material	Reaction conditions	Products			Ratio
		D-*xylo*	D-*arabino*	Elimination[a]	D-*xylo*/D-*arabino*
1	$LiAlH_4$/THF	**6**			1/0
1	nBu$_4$N$^+$H$_2$F$_3^-$	**7**	**8**		11.7/1
5	$LiAlH_4$/THF	**9**	**10**		1.8/1
5	nBu$_4$N$^+$H$_2$F$_3^-$	**11**	**12**	**13**	0.4/1[a]
5	KHF_2	**11**	**12**		1:1

[a]The elimination reaction product **13** was isolated in 56%.

couplings over one bond $^{1}J_{FC}$ ranged from 183 to 188 Hz. For the final di-*O*-acetates of 2- or 3-modified β-D-xylopyranoside **16–19,** some differences in conformation could be deduced. The $^{4}C_{1}$ conformation preponderates for derivatives **17–19** (indicated by the $^{3}J_{H,H}$ values). Compound **16** adopts the $^{1}C_{4}$ conformation, which is reflected also by a W-relationship between equatorial H-3 and the equatorial H-5 ($^{4}J_{3b,5b}$ 2.1 Hz).

2.2. Response of AcXEs to replacement of the OH-group in D-Xyl*p*βOMe diacetates

The rates of deacetylation of the acetylated derivatives of Xyl*p*βOMe by AcXE from *S. commune* (CE 1) and *T. reesei* (CE 5) as determined by chromatograms scanning are shown in Table 2. The data of an earlier study with the AcXE from *S. lividans*[12] are included for comparative purpose. AcXEs from families 1 and 5 do not show such dramatic changes in the rate of deacetylation of D-Xyl*p*βOMe diacetates at C-2 and C-3 due to replacement of the free OH-group by hydrogen or fluorine atoms as does *S. lividans* AcXE (Table 2). *S. lividans* AcXE is unable to accommodate in its substrate binding site two vicinal *trans*-related acetyl groups simultaneously. This is mainly the ability of *S. commune* AcXE. *T. reesei* AcXE showed an intermediate behavior.

Data in Table 2 for AcXE from *S. commune* more or less confirm our previous results obtained by GLC.[5–7] The best substrate for *S. commune* AcXE was found again to be 3,4-di-*O*-Ac-Xyl*p*βOMe. This diacetate was deacetylated approximately 3 times faster as compared to the fully acetylated derivative and 7 times faster than 2,4-di-*O*-Ac-Xyl*p*βOMe. Replacement of the OH-group at C-2 by hydrogen or fluorine in 3,4-di-*O*-Ac-Xyl*p*βOMe lowered the rate of deacetylation at C-3 by about 50%. Replacement of the OH-group at C-3 by hydrogen in 2,4-di-*O*-Ac-Xyl*p*βOMe lowered the rate of deacetylation at C-2 to 18%, while the presence of fluorine at C-2 increased the rate of deacetylation at C-3 by more than four times in comparison to the rate of deacetylation of 2,4-di-*O*-Ac-Xyl*p*βOMe. These data show that deacetylation at C-2 or C-3 by the *S. commune* AcXE is not influenced by the replacement of the vicinal OH-groups by hydrogen or fluorine so strongly as in the case of the other two AcXEs. The weaker role of the *trans* vicinal group on the rate of deacetylation is further supported by a relatively high rate and lower regioselectivity of deacetylation of 2,3,4-tri-*O*-Ac-Xyl*p*βOMe, 2,4-di-*O*-Ac-Xyl*p*βOMe and, particularly, 3,4-di-*O*-Ac-Xyl*p*βOMe, from which two major monoacetates are generated on initial deacetylation.[6] The regioselectivity of deacetylation of these derivatives by AcXE from *S. lividans* and *T. reesei* is very high and leads in all cases to high yields of 4-*O*-Ac-Xyl*p*βOMe.[5,7] The major differences between the three investigated AcXEs are illustrated by drawing the productive complexes with some of the substrates (Fig. 1).

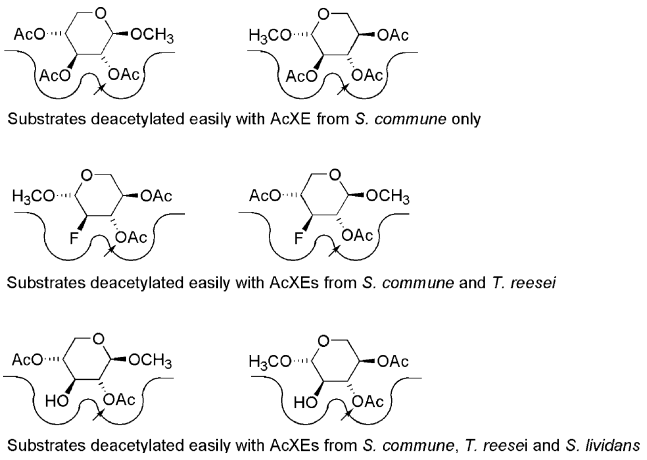

Figure 1. Scheme illustrating the main differences in structural requirements for the substrates of AcXEs belonging to three different CE families. The substrates are shown in hexagonal projections as recommended by Stick.[29] The target acetyl group marked with arrow and the *trans* vicinal group are filling the hypothetical enzyme-substrate binding sites. Note the reverse orientation of the binding of 2,3,4-tri-*O*-Ac- and 3,4-di-*O*-acetylated derivatives.

AcXE from *T. reesei* responded to structural changes in Xyl*p*βOMe diacetates with an intermediate behavior between the enzymes from *S. lividans* and *S. commune*. For *T. reesei* AcXE, fully acetylated Xyl*p*βOMe is 5 times worse substrate than 2,4-di-*O*-Ac-Xyl*p*βOMe and

Table 2.

Derivatives of D-Xyl*p*βOMe	AcXE *S. lividans*[a] CE family 4		AcXE *S. commune* CE family 1		AcXE *T. reesei* CE family 5	
	Specific activity nmol min^{-1} mg^{-1}	Rel. rate	Specific activity[b] nmol min^{-1} mg^{-1}	Rel. rate	Specific activity[b] nmol min^{-1} mg^{-1}	Rel. rate
2,3,4-tri-*O*-Ac	0.044	1.0	35.1	1.0	3.9	1.0
2,4-di-*O*-Ac	175	3923	15.3	0.43	19.6	5.0
3,4-di-*O*-Ac	34	771	113	3.22	6.6	1.7
3-deoxy-2,4-di-*O*-Ac (**16**)	0.063	1.4	2.8	0.08	0.47	0.12
3-deoxy-3-fluoro-2,4-di-*O*-Ac (**17**)	1.6	36.2	65.6	1.86	25.4	6.5
2-deoxy-3,4-di-*O*-Ac (**18**)	0.055	1.2	50.0	1.42	1.5	0.38
2-deoxy-2-fluoro-3,4-di-*O*-Ac (**19**)	0.16	4.7	63.3	1.80	8.7	2.2

[a] Data from Ref. 12.
[b] Average values from three determinations.

about 2 times worse substrate than 3,4-di-*O*-Ac-Xyl*p*βOMe. Already these data indicate that a free vicinal OH-group at positions 2 or 3 in the substrates plays an important role in the mechanism of deacetylation by the *T. reesei* enzyme. Deoxygenation at C-3 in 2,4-di-*O*-Ac-Xyl*p*βOMe leads to a 40-fold decrease of the rate of deacetylation at C-2. The same is partially true also for 3,4-di-*O*-Ac-Xyl*p*βOMe. Only deoxygenation at C-2 leads to a decrease in the deacetylation rate at C-3, but not fluorination. The hydroxy group seems to behave as a hydrogen bond acceptor since its absence is not only reversed by fluorine substitution, but the presence of the fluorine atom brings about a 30% increase in the deacetylation rate at both positions 2 and 3. It should be noted that the enhancing effect of the fluorine atom on the rate of deacetylation could also be ascribable to its electron withdrawing effect, increasing the electron deficiency on the acetyl group.

S. commune AcXE belonging to carbohydrate esterase family 1 does not require a free vicinal OH-group for its action so strongly as AcXE from *T. reesei* and, particularly, as AcXE from *S. lividans* for the action of which a vicinal OH-group is essential (Fig. 1). The vicinal OH-group in the deacetylation at C-2 or C-3 can be replaced by fluorine for *T. reesei* AcXE, but not for *S. lividans* AcXE. Consequently, the regioselectivity of deacetylation of the tested substrates by *S. commune* AcXE is lower than with the other two AcXEs. The obvious tolerance of the *S. commune* enzyme to an acetyl group esterifying the vicinal OH-group suggests that this enzyme is capable to operate on doubly acetylated xylopyranosyl residues of acetyl-4-*O*-methyl-D-glucuronoxylan more efficiently than AcXEs from *T. reesei* and *S. lividans*. The observed rates of deacetylation of the tested substrates by *S. commune* AcXE are not compatible with our earlier hypothesis introduced for AcXE from *S. lividans*[12] that deacetylation at C-2 and C-3 involves a common five-membered ring *ortho* ester intermediate. The dependence of the deacetylation rate at C-2 and C-3 on the presence of a free vicinal OH-group in AcXEs from *S. lividans* and *T. reesei* is seemingly in accord with the hypothesis of the formation of the five-membered *ortho* ester intermediate, however, based on the information on size of the substrate binding site of *T. reesei* AcXE[4] and *Penicillium purpurogenum* AcXE[3] (both CE 5), we are inclined to share the opinion that the acetylated derivatives of Xyl*p*βOMe and its deoxy and deoxyfluoro analogues are too small substrates to investigate the positional specificity of the enzymes.[4,20] The enzymes can form productive complexes with these small substrates in different orientations. Nevertheless, the prepared analogues of D-Xyl*p*βOMe diacetates enable to obtain insight into the mechanism of deacetylation, particularly to assess the role of the free vicinal OH-groups in the deacetylation mechanism.

3. Conclusion

The 2- and 3-deoxy and 2- and 3-deoxyfluoro analogues of acetylated derivatives of D-Xyl*p*βOMe were synthesized by routes that involves the regioselective nucleophilic epoxide ring opening of a 2,3-anhydropentopyranoside precursor. The rates of deacetylation of the acetylated derivatives of D-Xyl*p*βOMe, and their analogues, by AcXEs from *S. commune* and *T. reesei* were determined and compared with the performance of AcXE from *S. lividans*.[12] AcXEs are members of three different carbohydrate esterase families, 1, 5, and 4, respectively.[8,21] In accord with this fact, the enzymes showed a different behavior with respect to the character of the functional group *trans* vicinal to the deacetylated position 2 or 3. Substitution of the vicinal hydroxyl group by hydrogen hampered dramatically the deacetylation reaction by AcXEs from *S. lividans* and *T. reesei* but not that by AcXE from *S. commune*. The vicinal hydroxy group in the acetylated substrate cannot be replaced by fluorine in *S. lividans* AcXE, but this can occur with *T. reesei* AcXE. This result is not in favor of a deacetylation mechanism at C-2 and C-3 that would involve a five-membered *ortho* ester intermediate,[7] which still cannot be excluded in the case of AcXE from *S. lividans*. Deacetylation by AcXE from *S. commune* does not seem to be influenced significantly by the character of the functional group on the neighboring carbon atom. The enzyme tolerates well not only the absence of a vicinal OH-group, but also the presence of a vicinal acetoxy group. The overall results also support the view that studies of positional specificity of AcXEs may require substrates with a larger aglycon than a methyl group, an aglycon that would guarantee a proper orientation of the substrate into the substrate binding sites of AcXEs. Such substrates could be acetylated 4-nitrophenyl glycosides that have already been synthesized.[9,22]

4. Experimental

4.1. General methods

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 20 °C. Microanalyses were performed with a Fisons EA 1108 analyzer. ^{1}H and ^{13}C NMR spectra (internal standard Me_4Si) were recorded with a Bruker AM 300 spectrometer. Homonuclear correlation experiment COSY-45 and two-dimensional ^{1}H-^{13}C HMQC technique were used.[19] F NMR spectra were recorded with a Varian 300 spectrometer in acetone-d_6 and chemical shifts are given relative to CF_3CO_2H as external standard. Solvents were distilled from the appropriate drying agents before

use. All reactions were monitored by TLC on Silica Gel 60 (0.25 mm, E. Merck) and spraying the plates with 1% orcinol in 10% (v/v) EtOH soln of H_2SO_4 followed by heating at ca. 200 °C. Column chromatography was performed on Silica Gel 60 (100–160 mesh). Fully acetylated methyl β-D-xylopyranoside (2,3,4-tri-*O*-Ac-Xyl*p*βOMe) was prepared by acetylation of commercially available methyl β-D-xylopyranoside (Sigma) with Ac_2O in pyridine. Methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside (2,4-di-*O*-Ac-Xyl*p*βOMe) was a kind gift from Dr. Ján Hirsch. Methyl 3,4-di-*O*-acetyl-β-D-xylopyranoside (3,4-di-*O*-Ac-Xyl*p*βOMe) was prepared by enzymatic acetylation of methyl β-D-xylopyranoside with Lipase PS-30 (Amano).[23] Pure AcXE from *S. commune* was obtained as described previously.[6,24] Pure AcXE from *T. reesei* was a generous gift from Dr. Maija Tenkanen (University of Helsinki, Finland).

4.2. Epoxide ring opening of 2 with AcONa–AcOH

Sodium acetate (32.8 g, 400 mmol) was dissolved in AcOH (60 mL) with stirring at 100 °C and methyl 2,3-anhydro-4-*O*-benzyl-β-D-ribopyranoside[14] (9.44 g, 40 mmol) was added. The reaction mixture was stirred and heated to mild reflux for 3 h and then poured into ice-water and extracted with $CHCl_3$. The organic layer was washed twice with water, dried (Na_2SO_4), decolorized with charcoal, filtered, and concentrated to obtain a crude mixture of two products. The mixture was suspended into diethyl ether and the precipitated white crystals of 3-acetate **3** (6.16 g, 52%) were filtered. The concentrated filtrate was chromatographed (8:1 toluene–acetone) to give **3** (1.68 g, 14%) as a first fraction and the 2-acetate **4** as a second fraction.

4.2.1. Methyl 3-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranoside (3). White solid (7.84, 66%), mp 161–162 °C (from EtOAc); lit.[16a] mp 162–163 °C; $[\alpha]_D^{20}$ − 49 (*c* 1.0, $CHCl_3$); lit.[16a] $[\alpha]_D^{22}$ −48 (*c* 1.0, $CHCl_3$); R_f 0.27 (8:1 toluene–acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.28 (m, 5H, H-Ph), 5.05 (t, 1H, $J_{2,3}$ 7.9 Hz, H-3), 4.61 (s, 2H, CH_2), 4.28 (d, 1H, $J_{1,2}$ 6.3 Hz, H-1), 4.00 (dd, 1H, $J_{4,5a}$ 4.4, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.56 (dt, 1H, $J_{3,4}$ 8.0, $J_{4,5b}$ 8.6 Hz, H-4), 3.50 (s, 3H, OCH_3), 3.44 (ddd, 1H, H-2), 3.37 (dd, 1H, H-5b), 2.80 (d, 1H, $J_{H-OH,H-2}$ 5.2 Hz, H–OH), 2.08 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.8 (*C*OCH_3, 137.6, 2×128.5, 128.0, 2×127.7 (C–Ph), 103.8 (C-1), 74.6 (C-4), 74.0 (C-3), 72.7 (CH_2), 71.3 (C-2), 62.4 (C-5), 56.9 (OCH_3), 21.0 (CO*C*H_3). Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 61.08; H, 6.90.

4.2.2. Methyl 2-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranoside (4). Colorless oil (2.92 g, 25%), $[\alpha]_D^{20}$ −58 (*c* 1.0, $CHCl_3$); R_f 0.38 (8:1 toluene–acetone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.31 (m, 5H, H-Ph), 4.77 (dd, 1H, $J_{1,2}$ 6.7, $J_{2,3}$ 8.3 Hz, H-2), 4.67 (dd, 2H, CH_2), 4.33 (d, 1H, H-1), 3.99 (dd, 1H, $J_{4,5a}$ 4.6, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.74 (dt, 1H, $J_{3,4}$ 8.3 Hz, H-3), 3.52 (ddd, 1H, H-4), 3.45 (s, 3H, OCH_3), 3.30 (dd, 1H, $J_{4,5b}$ 8.7, H-5b), 2.71 (d, 1H, $J_{H-OH,H-3}$ 4.1 Hz, H–OH), 2.11 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.4 (*C*OCH_3), 137.8, 2×129.7, 127.8, 2×127.7 (C–Ph), 101.5 (C-1), 77.1 (C-4), 73.0 (C-3), 72.8 (CH_2), 72.7 (C-2), 62.7 (C-5), 56.4 (OCH_3), 20.8 (CO*C*H_3). Anal. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 61.20; H, 6.88.

4.3. Chemoenzymatic transformation of the 2-*O*-acetate 4 to the 3-*O*-acetate 3

Compound **4** (2.2 g, 7.4 mmol) was dissolved in a 0.7 M soln of MeONa in MeOH (50 mL) and the reaction was stirred at room temperature. After 5 h, the mixture was neutralized with Dowex 50 WX8 (H^+) resin, filtered, concentrated, and crystallized from i-PrOH–*n*-hexane to obtain methyl 4-*O*-benzyl-β-D-xylopyranoside (1.72 g, 91%); mp 98–100 °C; lit.[14] mp 99–100 °C. The resulting methyl 4-*O*-benzyl-β-D-xylopyranoside (1.5 g, 5.9 mmol) was dissolved in MeCN (100 mL); then vinyl acetate (2.5 mL) and Lipase PS-30 (9 g) were added. The reaction suspension was shaken at 35 °C and 200 rpm for 36 h. At the end of the reaction, the lipase powder was filtered off, the solvent was evaporated from the filtrate and the residue was triturated with diethyl ether. The precipitated white solid was separated by filtration. The yield after crystallization from EtOAc was 1.61 g (92 %). All physical and spectral characteristics were the same as for **3**.

4.4. Methyl 2,3-anhydro-4-*O*-benzyl-β-D-lyxopyranoside (5)

The 3-acetate **3** (7.40 g, 25 mmol) was dissolved in pyridine (60 mL) in the presence of a catalytic amount of DMAP. *p*-Toluenesulfonyl chloride (9.5 g, 50 mmol) was added at 0 °C and the mixture was stirred at room temperature. After 72 h, ice-water (60 mL) was added and the mixture was extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed with 1 N HCl, two times with water, dried (Na_2SO_4), and concentrated. The crude product (10.46 g, 23 mmol, 93%) was dissolved in 0.7 M MeONa (92 mL) and stirred overnight at room temperature. The soln was then neutralized with 1 M H_2SO_4 and extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed twice with water, dried (Na_2SO_4) and concentrated under diminished pressure. The crude syrup was purified by column chromatography (2:1 toluene–EtOAc) to afford epoxide **5** (5.13 g, 87%) as a colorless oil: $[\alpha]_D^{20}$ −74 (*c* 1.0, $CHCl_3$); lit.[23] $[\alpha]_D^{20}$ −77 (*c* 1.0, $CHCl_3$); R_f 0.50 (3:2 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.29 (m, 5H, H-Ph), 4.93 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 4.67 (dd, 2H, CH_2), 3.80 (dd, 1H, $J_{4,5a}$ 2.1, $J_{5a,5b}$ 12.1 Hz, H-5a), 3.78–

3.76 (m, 1H, H-4), 3.59 (dt, 1H, $J_{4,5b}$ 3.1, $J_{3,5b}$ 1.6 Hz, H-5b), 3.46 (s, 3H, OCH_3), 3.37 (dd, 1H, $J_{2,3}$ 3.7 Hz, H-2), 3.33 (dd, 1H, $J_{3,4}$ 1.9 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 137.6, 2×128.6, 128.1, 2×127.9 (C–Ph), 94.9 (C-1), 72.0 (CH_2), 70.4 (C-4), 58.6 (C-5), 55.8 (OCH_3), 51.5 (C-2), 50.5 (C-3). Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 65.61; H, 7.12.

4.5. Ring-opening of epoxide 1 by hydride reduction

Methyl 2,3-anhydro-β-D-ribopyranoside[13] (**1**) (0.73 g, 5 mmol) was dissolved in dry THF (10 mL), and the soln was added dropwise to a stirred suspension of $LiAlH_4$ (0.57 g, 15 mmol) in THF (15 mL) under nitrogen at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 48 h. Water (15 mL) was added dropwise upon cooling, the mixture was filtered through a pad of Celite and the residue was washed with EtOAc. The filtrate was concentrated to 1/5 vol and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated. There was no evidence for the presence of the 2-deoxy-*arabino* isomer in the ^1H NMR spectrum of the crude product; only about 5% of starting material was found. The crude product was purified by chromatography on silica gel using 9:1 $CHCl_3$–MeOH as eluent to afford **6**.

4.5.1. Methyl 3-deoxy-β-D-*erythro*-pentopyranoside (6). Colorless oil (0.55 g, 74%), $[\alpha]_D^{20}$ −106 (*c* 1.0, MeOH); R_f 0.45 (9:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, D_2O): δ 4.36 (dd, 1H, $J_{1,2}$ 6.1 Hz, H-1), 3.95 (ddd, 1H, $J_{4,5a}$ 4.1, $J_{5a,5b}$ 11.3 Hz, H-5a), 3.85 (dddd, 1H, $J_{3a,4}$ 4.4, $J_{3b,4}$ 9.1, $J_{4,5b}$ 7.8 Hz, H-4), 3.52 (ddd, 1H, $J_{2,3a}$ 4.4, $J_{2,3b}$ 9.1 Hz, H-2), 3.50 (s, 3H, OCH_3), 3.38 (dd, 1H, H-5b), 2.26 (ddt, 1H, $J_{3a,5b}$ 1.8 Hz, H-3a), 1.58 (dt, 1H, $J_{3a,3b}$ 12.8 Hz, H-3b); ^{13}C NMR ($CDCl_3$): δ 101.03 (C-1), 66.8 (C-2), 65.4 (C-4), 64.5 (C-5), 55.2 (OCH_3), 30.9 (C-3). Anal. Calcd for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.64; H, 8.14.

4.6. Ring opening of epoxide 1 by hydrofluorination

The epoxide **1** (0.73 g, 5 mmol), $Bu_4N^+H_2F_3^-$ (1.8 g, 6 mmol) and solid KHF_2 (0.94 g, 12 mmol) were stirred and heated at 130 °C until complete disappearance of the starting compound (12 h). The brown reaction mixture was cooled, diluted with EtOAc (120 mL) and washed with a satd aq $NaHCO_3$. After drying the organic layer with Na_2SO_4, filtration, and evaporation of the solvent, the crude residue was purified by chromatography using 10:1 $CHCl_3$–MeOH as eluent to give syrupy 3-deoxy-3-fluoro-β-D-xylopyranoside **7**, which solidified upon storage, and the 2-deoxy-2-fluoro-β-D-arabinopyranoside isomer **8**.

4.6.1. Methyl 3-deoxy-3-fluoro-β-D-xylopyranoside (7). White solid (0.58 g, 70%), mp 104–105 °C (from MeOH–Et_2O or $CHCl_3$); $[\alpha]_D^{20}$ −56 (*c* 1.0, MeOH); lit.[16c] mp 105 °C; R_f 0.35 (9:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 4.38 (ddd, 1H, $J_{2,3}$ 7.9, $J_{3,4}$ 8.1, $J_{3,F}$ 52.0 Hz, H-3), 4.22 (d, 1H, $J_{1,2}$ 6.9 Hz, H-1), 4.06 (ddd, 1H, $J_{4,5a}$ 5.6, $J_{5a,F}$ 5.8, $J_{5a,5b}$ 11.6 Hz, H-5a), 4.00–3.88 (m, 1H, H-4), 3.61 (ddd, 1H, $J_{2,F}$ 12.9 Hz, H-2), 3.55 (s, 3H, OCH_3), 3.30 (dd, 1H, $J_{4,5b}$ 9.4 Hz, H-5b), 2.58 (br s, 1H, OH), 2.42 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 103.5 (d, $J_{C\text{-}1,F}$ 10.0 Hz, C-1), 95.4 (d, $J_{C\text{-}3,F}$ 182.5 Hz, C-3), 71.6 (d, $J_{C\text{-}2,F}$ 18.3 Hz, C-2), 68.4 (d, $J_{C\text{-}4,F}$ 19.1 Hz, C-4), 63.7 (d, $J_{C\text{-}5,F}$ 7.4 Hz, C-5), 57.2 (OCH_3); ^{19}F NMR: δ −115.8 (ddt). Anal. Calcd for $C_6H_{11}FO_4$: C, 43.37; H, 6.67; F, 11.43. Found: C, 43.19; H, 6.92; F, 11.25.

4.6.2. Methyl 2-deoxy-2-fluoro-β-D-arabinopyranoside (8). Colorless oil (0.05 g, 6%), $[\alpha]_D^{20}$ −71 (*c* 1.0, MeOH); R_f 0.43 (9:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, D_2O): δ 4.67 (ddt, 1H, $J_{1,2}$ 4.1, $J_{2,F}$ 48.5 Hz, H-2), 4.60 (dd, 1H, $J_{1,F}$ 2.4 Hz, H-1), 4.11 (ddd, 1H, $J_{2,3}$ 6.8, $J_{3,4}$ 3.5, $J_{3,F}$ 11.1 Hz, H-3), 3.94–3.74 (m, 3H, H-4, H-5a, H-5b), 3.46 (s, 3H, OCH_3), 2.63 (br s, 2H, 2×OH); ^{19}F NMR: δ −123.6 (dm). Anal. Calcd for $C_6H_{11}FO_4$: C, 43.37; H, 6.67; F, 11.43. Found: C, 43.06; H, 7.02; F, 11.18.

4.7. Ring-opening of epoxide 5 by hydride reduction

Methyl 2,3-anhydro-4-*O*-benzyl-β-D-lyxopyranoside (**5**) (2.13 g, 9 mmol) was dissolved in dry THF (18 mL), and the soln was added dropwise to a stirred suspension of $LiAlH_4$ (1.14 g, 30 mmol) in THF (30 mL) under N_2 at 0 °C. The reaction mixture was allowed to warm up to room temperature and further stirred for 40 h. Water (20 mL) was added dropwise with cooling, the mixture was filtered through a pad of Celite and the residue was washed with CH_2Cl_2. The filtrate was concentrated to 1/4 vol and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated under diminished pressure. The resulting crude material was purified by column chromatography on silica gel using 2:1 toluene–EtOAc as eluent to obtain the 2-deoxy-β-D-xylopyranoside **9** and the 3-deoxy-β-D-arabinopyranoside analogue **10**.

4.7.1. Methyl 4-*O*-benzyl-2-deoxy-β-D-*threo*-pentopyranoside (9). White crystals (1.26 g, 53%), mp 36 °C (from i-Pr_2O–cyclohexane); $[\alpha]_D^{20}$ −132 (*c* 1.0, $CHCl_3$); R_f 0.35 (3:2 toluene–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.29 (m, 5H, H-Ph), 4.72 (dd, 1H, $J_{1,2a}$ 3.5, $J_{1,2b}$ 4.2 Hz, H-1), 4.65 (dd, 2H, J_{gem} 12.0 Hz, CH_2), 4.05 (dd, 1H, $J_{4,5a}$ 2.4, $J_{5a,5b}$ 12.6 Hz, H-5a), 3.87 (dt, 1H, $J_{2a,3}$ 3.5, $J_{2b,3}$ 4.2, $J_{3,4}$ 4.2 Hz, H-3), 3.58 (dd, 1H, $J_{4,5b}$ 3.6 Hz, H-5b), 3.45–3.36 (m, 1H, H-4), 3.43 (s, 3H, OCH_3), 2.27 (dt, 1H, $J_{2a,2b}$ 14.1 Hz, H-2a), 1.78 (dt, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 138.2, 128.5, 127.9, 127.8 (C–Ph), 99.4

(C-1), 76.0 (C-4), 71.6 (CH_2), 66.3 (C-3), 58.4 (C-5), 55.7 (OCH_3), 32.7 (C-2). Anal. Calcd for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.83; H, 7.64.

4.7.2. Methyl 4-*O*-benzyl-3-deoxy-β-D-*threo*-pentopyranoside (10). Colorless oil (0.70 g, 29%), $[\alpha]_D^{20}$ −114 (*c* 1.0, $CHCl_3$); R_f 0.27 (3:2 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.26 (m, 5H, H-Ph), 4.65 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 4.56 (s, 2H, CH_2), 4.02 (ddd, 1H, $J_{2,3a}$ 4.7 Hz, H-2), 3.70–3.59 (m, 3H, H-4, H-5a, H-5b), 3.45 (s, 3H, OCH_3), 2.07 (m, 1H, H-3a), 1.81 (ddd, 1H, $J_{2,3b}$ 11.0, $J_{3b,4}$ 2.9, $J_{3a,3b}$ 12.4 Hz, H-3b). Anal. Calcd for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.65; H, 7.92.

4.8. Ring-opening of epoxide 5 by hydrofluorination

Epoxide **5** (1.45 g, 6 mmol) and KHF_2 (7 g) in ethane-1,2-diol (28 mL) was heated for 3 h at 180 °C. After cooling, the suspension was neutralized with a satd aq $NaHCO_3$ and the neutral soln was extracted with $CHCl_3$ (3 × 70 mL), the extract was dried with Na_2SO_4, filtered, and evaporated to dryness under diminished pressure. The residue was purified by column chromatography (1:0 → 1:1 toluene–EtOAc) to give first the 2-deoxy-2-fluoro-β-D-xylopyranoside derivative **11** and next the 3-deoxy-3-fluoro-β-D-arabinopyranoside derivative **12**.

4.8.1. Methyl 4-*O*-benzyl-2-deoxy-2-fluoro-β-D-xylopyranoside (11). White crystals (0.54 g, 36%), mp 84–85 °C (from i-Pr_2O–cyclohexane); $[\alpha]_D^{20}$ −44 (*c* 1.0, $CHCl_3$); R_f 0.51 (3:2 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.31 (m, 5H, Ph), 4.67 (dd, 2H, J_{gem} 11.8 Hz, CH_2), 4.34 (dd, 1H, $J_{1,2}$ 7.2, $J_{1,F}$ 3.7 Hz, H-1), 4.15 (ddd, 1H, $J_{2,3}$ 8.5, $J_{2,F}$ 50.5 Hz, H-2), 3.94 (ddd, 1H, $J_{4,5a}$ 5.0, $J_{5a,5b}$ 11.7 Hz, H-5a), 3.82 (ddd, 1H, $J_{3,4}$ 8.4, $J_{3,F}$ 15.2 Hz, H-3), 3.52 (s, 3H, OCH_3), 3.57–3.41 (m, 1H, H-4), 3.23 (dd, 1H, $J_{4,5b}$ 9.7 Hz, H-5b), 3.06 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 137.7, 128.5, 128.0, 127.8 (C–Ph), 101.5 (d, $J_{C\text{-}1,F}$ 23.4 Hz, C-1), 91.4 (d, $J_{C\text{-}2,F}$ 185.4 Hz, C-2), 76.8 (d, $J_{C\text{-}4,F}$ 7.0 Hz, C-4), 74.0 (d, $J_{C\text{-}3,F}$ 18.4 Hz, C-3), 73.1 (CH_2), 63.3 (C-5), 56.8 (OCH_3); ^{19}F NMR: δ −121.0 (dd). Anal. Calcd for $C_{13}H_{17}FO_4$: C, 60.93; H, 6.69; F, 7.41. Found: C, 61.18; H, 6.79; F, 7.28.

4.8.2. Methyl 4-*O*-benzyl-3-deoxy-3-fluoro-β-D-arabinopyranoside (12). Colorless oil (0.53 g, 35%), $[\alpha]_D^{20}$ −168 (*c* 1.0, $CHCl_3$); R_f 0.30 (3:2 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.28 (m, 5H, Ph), 4.84 (dd, 1H, $J_{1,2}$ 3.8, $J_{1,F}$ 3.9 Hz, H-1), 4.72 (dd, 2H, J_{gem} 12.2 Hz, CH_2), 4.68 (ddd, 1H, $J_{2,3}$ 9.4, $J_{3,4}$ 3.4, $J_{3,F}$ 48.4 Hz, H-3), 4.22 (dt, 1H, $J_{2,F}$ 10.8 Hz, H-2), 3.93–3.91 (m, 1H, H-4), 3.74 (ddd, 1H, $J_{5a,F}$ 2.6, $J_{4,5a}$ 6.3 Hz, H-5a), 3.66 (d, 1H, $J_{4,5b}$ ~0, $J_{5a,5b}$ 12.5 Hz, H-5b), 3.43 (s, 3H, OCH_3), 2.11 (br s, 1H, OH); ^{19}F NMR: δ −124.8 (dm). Anal. Calcd for $C_{13}H_{17}FO_4$: C, 60.93; H, 6.69; F, 7.41. Found: C, 61.14; H, 7.02; F, 7.22.

4.9. Methyl 4-*O*-benzyl-3-deoxy-α-L-glycero-pent-3-enopyranoside (13)

The epoxide **5** (1.77 g, 7.5 mmol), $Bu_4N^+H_2F_3^-$ (4.52 g, 15 mmol) and KHF_2 (2.34 g, 30 mmol) were stirred and heated for 25 h at 135 °C. The dark reaction mixture was cooled, diluted with $CHCl_3$ (200 mL), and washed with a satd aq $NaHCO_3$, and brine. After drying the organic layer with Na_2SO_4, filtration and evaporation of the solvent, the residue was chromatographed on silica gel using toluene, then 2:1 toluene–EtOAc as eluent to give successively the elimination product **13** (0.99 g, 56%), the 3-deoxy-3-fluoro-β-D-arabinopyranoside derivative **12** (0.29 g, 15%) and the 2-deoxy-2-fluoro-β-D-xylopyranoside derivative **11** (0.11 g, 6%) and **13**: mp 54–55 °C (from i-Pr_2O–cyclohexane); $[\alpha]_D^{20}$ −127 (*c* 1.0, $CHCl_3$); R_f 0.36 (3:2 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.29 (m, 5H, H-Ph), 4.84 (dt, 1H, $J_{2,3}$ 3.4, $J_{3,5a}$ 1.3, $J_{3,5b}$ 1.3 Hz, H-3), 4.78 (dt, 2H, J_{gem} 15.2 Hz, CH_2), 4.66 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.34–4.28 (m, 1H, H-2), 4.15 (dt, 1H, $J_{5a,CH2}$ 1.4, $J_{5a,5b}$ 15.2 Hz, H-5a), 3.98 (dt, 1H, $J_{5b,CH2}$ 1.2 Hz, H-5b), 3.54 (s, 3H, OCH_3), 2.21 (d, 1H, $J_{2,OH}$ 9.1 Hz, H–OH); ^{13}C NMR ($CDCl_3$): δ 154.2 (C-4), 136.2, 128.5, 128.1, 127.7 (C–Ph), 98.7 (C-1), 94.4 (C-3), 69.2 (CH_2), 64.8 (C-2), 60.5 (C-5), 56.2 (OCH_3). Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.23; H, 7.06.

4.10. Methyl 2-deoxy-β-D-*threo*-pentopyranoside (14)

Compound **9** (1.20 g, 5 mmol) was dissolved in MeOH (50 mL) and 10% Pd/C (0.25 g) was added. The reaction mixture was intensively stirred under H_2 overnight. Filtration through Celite, concentration of the filtrate and purification by flash chromatography (9:1 $CHCl_3$–MeOH) gave pure **14** (0.70 g, 95%) as a white solid: mp 82–84 °C (from EtOAc–cyclohexane); lit.[25] mp 83–84 °C; $[\alpha]_D^{20}$ −97 (*c* 1.0, MeOH); lit.[25] $[\alpha]_D^{15}$ −167 (*c* 1.0, $CHCl_3$); R_f 0.25 (9:1 $CHCl_3$–MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 4.45 (dd, 1H, $J_{1,2a}$ 2.4, $J_{1,2b}$ 8.0 Hz, H-1), 3.92 (dd, 1H, $J_{4,5a}$ 4.5, $J_{5a,5b}$ 11.6 Hz, H-5a), 3.53 (ddd, 1H, $J_{2a,3}$ 4.8, $J_{2b,3}$ 9.9, $J_{3,4}$ 7.7 Hz, H-3), 3.41 (s, 3H, OCH_3), 3.39 (ddd, 1H, $J_{4,5b}$ 8.5 Hz, H-4), 3.18 (dd, 1H, H-5b), 2.11 (ddd, 1H, $J_{2a,2b}$ 13.0 Hz, H-2a), 1.47 (ddd, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 102.4 (C-1), 72.0 (C-4), 71.5 (C-3), 65.9 (C-5), 56.6 (OCH_3), 38.4 (C-2). Anal. Calcd for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.53; H, 8.28.

4.11. Methyl 2-deoxy-2-fluoro-β-D-xylopyranoside (15)

Compound **11** (0.50 g, 2 mmol) was dissolved in MeOH (20 mL) and 10% Pd/C (0.1 g) was added. The suspension was hydrogenated as described for **14**. Purification by flash chromatography gave pure **15** (0.31 g, 96%) as white crystals: mp 105–107 °C (from i-Pr_2O–i-PrOH);

$[\alpha]_D^{20}$ −56 (*c* 1.0, MeOH); R_f 0.26 (9:1 $CHCl_3$–MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 4.60 (dd, 1H, $J_{1,2}$ 7.5, $J_{1,F}$ 2.7 Hz, H-1), 4.11 (ddd, 1H, $J_{2,3}$ 8.2, $J_{2,F}$ 50.9 Hz, H-2), 3.98 (dd, 1H, $J_{4,5a}$ 5.1, $J_{5a,5b}$ 11.2 Hz, H-5a), 3.74 (ddd, 1H, $J_{3,4}$ 8.7, $J_{3,F}$ 14.8 Hz, H-3), 3.70–3.62 (m, 1H, H-4), 3.56 (s, 3H, OCH_3), 3.35 (dd, 1H, $J_{4,5b}$ 10.4, H-5b); ^{13}C NMR (CD_3OD): δ 57.1 (OCH_3), 66.9 (C-5), 71.0 (d, $J_{C-4,F}$ 7.6 Hz, C-4), 76.4 (d, $J_{C-3,F}$ 17.4 Hz, C-3), 93.6 (d, $J_{C-2,F}$ 184.9 Hz, C-2), 103.4 (d, $J_{C-1,F}$ 23.4 Hz, C-1); ^{19}F NMR: δ −121.4 (dd). Anal. Calcd for $C_6H_{11}FO_4$: C, 43.37; H, 6.67; F, 11.43. Found: C, 43.68; H, 6.78; F, 11.38.

4.12. Acetylation of 6, 7, 14, and 15

Compounds **6**, **7**, **14** or **15** were dissolved in pyridine (0.9 mL per mmol glycoside) in the presence of a catalytic amount of DMAP. Ac_2O (0.8 mL per mmol glycoside) was added with cooling at 0 °C. The mixture was stirred at room temperature for 48 h. Then the soln was poured onto crushed ice, stirred for 2 h and extracted with CH_2Cl_2 (3 × 10 mL per mmol glycoside). The extract was washed with water, dried (Na_2SO_4), and after concentration and flash chromatography (2:1 toluene–EtOAc) pure **16**, **17**, **18** or **19** were obtained.

4.12.1. Methyl 2,4-di-*O*-acetyl-3-deoxy-β-D-*erythro*-pentopyranoside (16). The acetylation was performed with **6** (0.5 g, 3.4 mmol) to give **16** (0.72 g, 91%) as a colorless syrup: $[\alpha]_D^{20}$ −96 (*c* 1.0, $CHCl_3$); lit.[26] $[\alpha]_D$ −94 (*c* 1.0, $CHCl_3$); R_f 0.59 (1:1 toluene–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 4.84–4.80 (m, 1H, H-4), 4.74 (ddd, 1H, $J_{1,2}$ 1.7, $J_{2,3a}$ 4.0, $J_{2,3b}$ 1.2 Hz, H-2), 4.59 (d, 1H, H-1), 3.96 (dd, 1H, $J_{4,5a}$ 2.4, $J_{5a,5b}$ 12.7 Hz, H-5a), 3.68 (dt, 1H, $J_{4,5b}$ 2.3, $J_{3b,5b}$ 2.1 Hz, H-5b), 2.20 (dt, 1H, $J_{3a,4}$ 4.0, $J_{3a,3b}$ 15.4 Hz, H-3a), 3.43 (s, 3H, OCH_3), 2.11 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.04 (ddt, 1H, $J_{3b,4}$ 1.2 Hz, H-3b); ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0 (2 *C*OCH_3), 98.1 (C-1), 66.9 (C-2), 65.7 (C-4), 61.2 (C-5), 55.3 (OCH_3), 26.9 (C-3), 21.2, 21.1 (2 CO*C*H_3). Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.74; H, 6.86.

4.12.2. Methyl 2,4-di-*O*-acetyl-3-deoxy-3-fluoro-β-D-xylopyranoside (17). The reaction was carried out with **7** (0.55 g, 3.3 mmol) to obtain **17** (0.73 g, 89%) as white needles: mp 80–81 °C (from i-Pr_2O); $[\alpha]_D^{20}$ −73 (*c* 1.0, $CHCl_3$); R_f 0.52 (1:1 toluene–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 5.06–4.99 (m, 2H, H-2, H-4), 4.57 (ddd, 1H, $J_{2,3} = J_{3,4}$ 7.9, $J_{3,F}$ 50.4 Hz, H-3), 4.37 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 4.16 (dt, 1H, $J_{4,5a}$ 4.9, $J_{5a,F}$ 5.0, $J_{5a,5b}$ 12.0 Hz, H-5a), 3.47 (s, 3H, OCH_3), 3.32 (dd, 1H, $J_{4,5b}$ 8.2 Hz, H-5b), 2.13 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 169.7, 169.3 (2*C*OCH_3), 101.0 (d, $J_{C-1,F}$ 8.2 Hz, C-1), 89.7 (d, $J_{C-3,F}$ 187.9 Hz, C-3), 70.4 (d, $J_{C-2,F}$ 20.6 Hz, C-2), 68.9 (d, $J_{C-4,F}$ 20.7 Hz, C-4), 60.8 (d, $J_{C-5,F}$ 5.7 Hz, C-5), 56.6 (OCH_3), 20.8 (2CO*C*H_3); ^{19}F NMR: δ −117.0 (ddd). Anal. Calcd for $C_{10}H_{15}FO_6$: C, 48.00; H, 6.04; F, 7.59. Found: C, 48.11; H, 6.12; F, 7.48.

4.12.3. Methyl 3,4-di-*O*-acetyl-2-deoxy-β-D-*threo*-pentopyranoside (18). The reaction of acetylation was run with **14** (0.5 g, 3.4 mmol) and pure **18** was obtained as a colorless syrup (0.71 g, 90%): $[\alpha]_D^{20}$ −114 (*c* 1.0, $CHCl_3$); lit.[25] $[\alpha]_D^{20}$ −101 (*c* 1.0, $CHCl_3$); lit.[27] $[\alpha]_D^{19}$ −121.9 (*c* 1.0, $CHCl_3$); R_f 0.48 (1:1 toluene–EtOAc); ^1H NMR (400 MHz, $CDCl_3$): δ 4.94 (ddd, 1H, $J_{2a,3}$ 4.8, $J_{2b,3}$ 8.4, $J_{3,4}$ 6.9 Hz, H-3), 4.83 (ddd, 1H, $J_{4,5a}$ 4.0 Hz, $J_{4,5b}$ 6.7 Hz, H-4), 4.53 (dd, 1H, $J_{1,2a}$ 3.2, $J_{1,2b}$ 6.4 Hz, H-1), 4.11 (dd, 1H, $J_{5a,5b}$ 12.2 Hz, H-5a), 3.43 (s, 3H, OCH_3), 3.39 (dd, 1H, H-5b), 2.24 (ddd, 1H, H-2a), 2.07 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 1.76 (ddd, 1H, $J_{2a,2b}$ 13.7 Hz, H-2b); ^{13}C NMR ($CDCl_3$): δ 170.2 (*C*OCH_3), 170.1 (*C*OCH_3), 99.3 (C-1), 69.0 (C-4), 68.4 (C-3), 60.8 (C-5), 56.1 (OCH_3), 33.1 (C-2), 21.06 (CO*C*H_3), 20.9 (CO*C*H_3). Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.65; H, 7.08.

4.12.4. Methyl 3,4-di-*O*-acetyl-2-deoxy-2-fluoro-β-D-xylopyranoside (19). The reaction was carried out with **15** (0.25 g, 1.5 mmol) yielding **19** (0.35 g, 93%) after isolation by the usual manner: mp 101–102 °C (from i-Pr_2O); $[\alpha]_D^{20}$ −14 (*c* 1.0, $CHCl_3$); R_f 0.54 (1:1 toluene–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 5.28 (ddd, 1H, $J_{2,3}$ 8.0, $J_{3,4}$ 8.6, $J_{3,F}$ 13.9 Hz, H-3), 4.92 (ddd, 1H, $J_{4,5a}$ 5.0, $J_{4,5b}$ 8.9 Hz, H-4), 4.48 (dd, 1H, $J_{1,2}$ 6.5, $J_{1,F}$ 5.2 Hz, H-1), 4.26 (ddd, 1H, $J_{2,F}$ 49.5 Hz, H-2), 4.09 (dd, 1H, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.55 (s, 3H, OCH_3), 3.39 (dd, 1H, H-5b), 2.11 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 169.9 (2 *C*OCH_3), 101.3 (d, $J_{C-1,F}$ 24.2 Hz, C-1), 88.8 (d, $J_{C-2,F}$ 187.9 Hz, C-2), 71.3 (d, $J_{C-3,F}$ 21.4 Hz, C-3), 68.6 (d, $J_{C-4,F}$ 6.0 Hz, C-4), 62.0 (C-5), 56.9 (OCH_3), 20.7 (2 CO*C*H_3); ^{19}F NMR: δ −121.5 (ddd). Anal. Calcd for $C_{10}H_{15}FO_6$: C, 48.00; H, 6.04; F, 7.59. Found: C, 47.89; H, 6.22; F, 7.46.

4.13. Deacetylation of 2,3,4-tri-*O*-Ac-Xyl*p*βOMe, 2,4-di-*O*-Ac-Xyl*p*βOMe, 3,4-di-O-Ac-Xyl*p*βOMe, 16–19 with AcXEs and products analysis

Substrate solns (10 mM) in 0.1 M sodium phosphate buffer (pH 6.0) were incubated with the appropriate amount of purified AcXEs at 40 °C. Aliquots of the reaction mixtures were analyzed by TLC. Solns of 2,4-di-*O*-Ac-Xyl*p*βOMe and 3,4-di-*O*-Ac-Xyl*p*βOMe, which undergo spontaneous acetyl group migration in aqueous media, were always prepared freshly in the shortest possible time before enzyme addition and their enzymatic treatment was never longer than 15 min. Reaction mixtures of all substrates were analyzed by TLC on glass plates of Silicagel G-60 (E. Merck) in 2:1:0.1 EtOAc–

benzene–2-propanol as described in details elsewhere[12] The detected chromatograms[28] were scanned in a reflectance mode and the images were analyzed by densitometry using Un-Scan-It software (Silk Scientific Co., Orem, UT, USA). The rate of disappearance of the substrate, was used to estimate the rate of the first deacetylation step. The rate was referred to 1 mg of the protein, thus specific activities of the enzyme to individual substrates were calculated. The number of products as a result of the first deacetylation step was evaluated on TLC visually.

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