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Synthesis of acylated methyl β-D-xylopyranosides and their enzymic deacylations by rabbit serum esterases

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Abstract

Selective pivaloylations of methyl β-D-xylopyranoside have been studied under various reaction conditions. Partially pivaloylated products were submitted to additional acetylations. The structures were established by ^{1}H NMR spectroscopy. Representatives of acylated methyl β-D-xylopyranosides (acyl being pivaloyl, acetyl, or a combination of both) were submitted to hydrolysis catalyzed by rabbit serum and esterases isolated from rabbit serum. © 1997 Elsevier Science Ltd.

Keywords: Methyl β-D-xylopyranosides, acylated; Enzymic deacylations; Rabbit serum esterases

1. Introduction

In recent years the use of enzymes as catalysts in organic synthesis has developed into a very interesting field of research [1,2]. Enzymes can be used as stereoselective [2], chemoselective [3,4], and regioselective catalysts [1,5–7]. Due to the synthetic challenge that multifunctional sugars offer, enzymic techniques for the selective introduction of blocking groups into carbohydrates and/or their subsequent removal have been developed [5–7].

In a continuation of our work on enzymic regioselective deacylations of sugars with esterases from mammalian sera [8–18], we wish to report the syntheses of a series of pivaloylated and acetylated methyl β-D-xylopyranosides and their use as substrates for esterases in rabbit serum. The ability of pivaloyl chloride to acylate sugars selectively has been exploited in the synthesis of various pivaloylated simple glycosides [10,14,17] and disaccharides [19,20]. Chemical deacylation of both, pivaloylated and acetylated sugars can be achieved in basic conditions, unsuitable for base labile compounds. To avoid this, enzymic techniques were developed. Thus, it was shown that *O*-acyl derivatives of D-glucose [9,14,15,18], 2-acetamido-2-deoxy-D-glucose [10,11], and D-mannose [17] are good substrates for esterases in mammalian sera. Furthermore, the esterases from rabbit serum [13,16] and guinea-pig serum [18] with specificity for sugar substrates were isolated, partially purified, and their catalytic properties determined.

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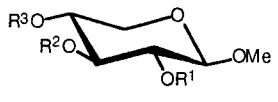
2. Results and discussion

Preparation of methyl O-acyl-β-D-xylopyranosides.—Various *O*-pivaloyl derivatives were prepared by esterification of methyl β-D-xylopyranoside (**1**) with different equivalents of pivaloyl chloride in pyridine. Acetylation was achieved by using an excess of acetic anhydride–pyridine mixture.

Thus, the reaction of **1** with 5 equivalents of pivaloyl chloride for 48 h produced the tripivalate **8** as the major product (63%). Some 2,4- (**6**, 7%) and 3,4-dipivalates (**7**, 19%) were isolated as well. Raising the temperature to 60 °C and leading the reaction for 24 h gave exclusively the tripivalate **8** (90%). The course of the reaction was monitored by TLC, and it was noticed that the first formed product was the dipivalate **7** followed by **6**. The dipivalate **6** disappears first from the reaction mixture followed by **7** to give **8** as the sole reaction product.

Acetylation of dipivalates **6** and **7** produced the monoacetylated products **17** and **18**, respectively.

Treatment of **1** with 3 equivalents of pivaloyl chloride for 2 h resulted in a multitude of products, some of which could be separated by column chromatography on silica gel. Thus, the 2,4-dipivalate **6** and the 3,4-dipivalate **7** were isolated in 25% and 26% yields, respectively. Only traces of the 2,3-dipivalate **5** were formed, but, nevertheless, could be isolated (1%) and identified by ^{1}H NMR spectroscopy and acetylation. Acetylation produced the monoacetylated product **16**. Small quantities of the tripivalate **8** (7%) were also isolated.



1	R_1, R_2, R_3 = H	**10***	R_1, R_3 = Ac; R_2 = H
2	R_1 = Piv; R_2, R_3 = H	**11**	R_1, R_2, R_3 = Ac
3	R_2 = Piv; R_1, R_3 = H	**12**	R_1 = Piv; R_2, R_3 = Ac
4	R_3 = Piv; R_1, R_2 = H	**13***	R_1 = Ac; R_2 = Piv; R_3 = H
5	R_1, R_2 = Piv; R_3 = H	**14**	R_2 = Piv; R_1, R_3 = Ac
6	R_1, R_3 = Piv; R_2 = H	**15**	R_3 = Piv; R_1, R_2 = Ac
7	R_2, R_3 = Piv; R_1 = H	**16**	R_1, R_2 = Piv; R_3 = Ac
8	R_1, R_2, R_3 = Piv	**17**	R_1, R_3 = Piv; R_2 = Ac
9*	R_1, R_2 = Ac; R_3 = H	**18**	R_2, R_3 = Piv; R_1 = Ac

* Compounds prepared by enzymic hydrolysis

Treatment of **1** with 1 equivalent of pivaloyl chloride for 45 min, followed by column chromatography on silica gel gave the 3-monopivalate **3** and the 4-monopivalate **4** in 20% and 22% yields, respectively. The 2-monopivalate **2** was formed in traces, and could not be separated from **4**. ^{1}H NMR analysis of the mixture of **2** and **4** showed that a 5:1 mixture of **4** and **2** was obtained. Some unreacted **1** (28%) was recovered from the reaction mixture as well.

Acetylation of the monopivalates **3** and **4** gave the diacetylated products **14** and **15**, respectively. For identification purposes the mixture of **2** and **4** was acetylated to give the mixture of diacetylated products **13** and **15**. Furthermore, the starting compound **1** was peracetylated to produce the triacetate **11** [21].

Enzymic hydrolyses.—Hydrolyses of the *O*-pivaloyl derivatives **6**, **7**, and **8**, the peracetylated compound **11**, and the *O*-acetyl-*O*-pivaloyl derivatives **14**, **15**, **17**, and **18** were catalyzed by whole rabbit serum (S) and by an esterase fraction isolated from rabbit serum (E) [13]. To exclude the possibility of some spontaneous hydrolysis effected by the reaction medium, control reactions were carried out parallel to every enzymic hydrolysis. Controls contained all reactants except the enzyme.

Thus, hydrolysis of the 3,4-dipivalate **7** with whole rabbit serum produced the mixture of 3- (**3**) and 4-monopivalates (**4**) in 33% and 41% yields, respectively. When isolated esterase was used the percentages were reversed, and **3** and **4** were formed in 44% and 32% yields, respectively. The reactions were stopped when all of the starting compound had disappeared.

Under identical experimental conditions, with both the native serum and the esterase fraction, the 2,4-dipivalate **6** underwent, in the first step, a partial intramolecular transesterification to give the 3,4-dipivalate **7**. In the second step **7** was hydrolyzed, to a limited extent, to give the expected monopivalates **3** and **4**. Thus, column chromatography yielded the unreacted **6** (S, 30%; E, 35%), dipivalate **7** (S, 24%; E, 22%), and the mixture of monopivalates **3** and **4** (S, 19%; E, 24%). The intramolecular transesterification was also noted in control reactions which indicates that it was not caused by the enzyme but most probably by the reaction medium. However, deacylation was not noted in controls, indicating that it was catalyzed by the enzyme. Some enzyme inhibition occurred during the reaction and only a small quantity of products was formed.

The tripivalate **8** was not hydrolyzed either by the whole serum or by the isolated esterase. On the contrary, its triacetate analogue **9** underwent regioselective deacetylation, both by rabbit serum and the esterase, to produce the 2,4-diacetate **10** in 58% and 53% yields, respectively. The reaction was stopped when major selectivity was observed.

Hydrolysis of methyl 2,4-di-*O*-acetyl-3-*O*-pivaloyl-β-D-xylopyranoside **14** resulted in the preferential hydrolysis of the pivaloyl group. Thus, both serum- and esterase-catalyzed hydrolyses produced the 2,4-diacetate **10** in 55% and 64% yields, respectively.

Enzymic hydrolysis of methyl 2,3-di-*O*-acetyl-4-*O*-pivaloyl-β-D-xylopyranoside **15** by serum and the esterase resulted in the preferential hydrolysis of the pivaloyl group as well, and the 2,3-diacetate **9** was formed in 16% and 27% yields, respectively. It was noted that, although no other products were formed, the reaction stops after 24 h. Changing reaction conditions gave no results and unreacted **15** was recovered in 52% (S) and 42% (E) yields.

Analogous to the tripivalate **8**, 3-*O*-acetyl-2,4-di-*O*-pivaloyl-β-D-xylopyranoside **17** was not hydrolyzed either by serum or the esterase.

Hydrolyses of methyl 2-*O*-acetyl-3,4-di-*O*-pivaloyl-β-D-xylopyranoside **18** by the serum and the esterase produced methyl 2-*O*-acetyl-3-*O*-pivaloyl-β-D-xylopyranoside **13** in 55% and 57% yields, respectively. Since **13** was not obtained by acylation it was acetylated for additional identification to give the diacetate **14**.

In conclusion it can be observed that partially acylated **6**, and completely acylated **8** and **17** do not undergo enzymic deesterification. All three compounds have a large pivaloyl group at *O*-2 in the sugar ring, and the possible explanation for their unreactivity might be the impossibility of such compounds to enter the enzyme active site. On the contrary, compounds acylated by a combination of acetyl and pivaloyl groups with an acetyl group at *O*-2 (**14**, **15**, **18**) hydrolyze in good yields and in a chemoselective manner. In all three cases a pivaloyl group is removed preferentially. In addition, when chemoselectivity is not possible as in peracetylated **11**, regioselective hydrolysis occurs, and the acetyl group at *O*-3 has been removed preferentially. Furthermore, it was noted that the 3,4-dipivalate **7** undergoes unselective and uncomplete hydrolysis to yield monopivalates. Both **7** and **11** have a smaller group (OH or OAc) at C-2 in the sugar ring.

All compounds were identified by their chromatographic mobilities and ^{1}H NMR spectra. On the basis of chemical shifts of pivaloyl and acetyl groups, chemical shifts and splitting pattern of ring protons, and previously reported data for the peracetylated and perbenzoylated α- and β-D-xylopyranosides [22], it was possible to determine the position of substituents on the sugar ring.

3. Experimental

General methods.—Column chromatography was performed on silica gel (Merck) and TLC on Kieselgel G (Merck) with solvent A, EtOAc–benzene (proportions are given in the text). The detection was effected by charring with H_2SO_4. ^{1}H NMR spectra (300 MHz, $CDCl_3$, internal Me_4Si) were recorded with a Varian Gemini spectrometer, and the data are given in Tables 1 and 2. Optical rotations were

Table 1
300 MHz ^{1}H NMR data ($CDCl_3$) for methyl *O*-acyl-β-D-xylopyranosides

Compd	Chemical shifts δ (ppm), *J* (Hz)					
	H-1 (d)	H-2 (m)	H-3 (t)	H-4 (m)	H-5′ (dd)	H-5 (dd)
2	4.47, $J_{1,2}$ 4.76	4.73	3.71 (m)	3.80	3.38 (m)	4.10 (m)
3	4.28, $J_{1,2}$ 6.45	3.55	4.76, *J* 8.11	3.77	3.36, $J_{5,5'}$ 11.79, $J_{4,5'}$ 8.77	4.06, $J_{5,5'}$ 11.79, $J_{4,5}$ 4.78
4	4.38, $J_{1,2}$ 5.38	3.48	3.75, *J* 6.78	4.81	3.40, $J_{5,5'}$ 12.21, $J_{4,5'}$ 6.78	4.09, $J_{5,5'}$ 12.18, $J_{4,5}$ 4.18
5	4.35, $J_{1,2}$ 6.19	4.91	4.91 (m)	3.80	3.38, $J_{5,5'}$ 11.78, $J_{4,5'}$ 8.69	4.09, $J_{5,5'}$ 11.72, $J_{4,5}$ 4.81
6	4.40, $J_{1,2}$ 6.20	4.81	3.78, *J* 7.79	4.81	3.36, $J_{5,5'}$ 11.90, $J_{4,5'}$ 7.71	4.08, $J_{5,5'}$ 12.00, $J_{4,5}$ 4.70
7	4.28, $J_{1,2}$ 6.90	3.50	5.12, *J* 8.60	4.93	3.33, $J_{5,5'}$ 12.04, $J_{4,5'}$ 9.76	4.10, $J_{5,5'}$ 11.60, $J_{4,5}$ 5.20
8	4.35, $J_{1,2}$ 7.42	4.99	5.30, *J* 9.25	4.99	3.32, $J_{5,5'}$ 11.48, $J_{4,5'}$ 9.76	4.11, $J_{5,5'}$ 11.46, $J_{4,5}$ 5.36
9	4.25, $J_{1,2}$ 7.02	4.90	5.09 (m)	3.80	3.34 (m)	4.11 (m)
10	4.38, $J_{1,2}$ 6.51	4.90	3.81 (m)	4.90	3.37, $J_{5,5'}$ 11.80, $J_{4,5'}$ 8.72	4.09, $J_{5,5'}$ 11.93, $J_{4,5}$ 4.79
11	4.40, $J_{1,2}$ 6.66	4.96	5.17, *J* 8.55	4.96	3.38, $J_{5,5'}$ 11.82, $J_{4,5'}$ 8.73	4.13, $J_{5,5'}$ 11.81, $J_{4,5}$ 5.22
12	4.40, $J_{1,2}$ 4.80	4.93	5.21 (m)	4.93	3.34 (m)	4.10 (m)
13	4.37, $J_{1,2}$ 6.52	4.95	4.85, *J* 8.14	3.78	3.37, $J_{5,5'}$ 11.76, $J_{4,5'}$ 9.08	4.10, $J_{5,5'}$ 11.87, $J_{4,5}$ 4.56
14	4.40, $J_{1,2}$ 6.74	4.97	5.16, *J* 8.70	4.97	3.39, $J_{5,5'}$ 11.76, $J_{4,5'}$ 8.95	4.11, $J_{5,5'}$ 11.57, $J_{4,5}$ 5.05
15	4.39, $J_{1,2}$ 6.90	4.89	5.20, *J* 8.67	4.89	3.37, $J_{5,5'}$ 11.58, $J_{4,5'}$ 8.99	4.12, $J_{5,5'}$ 11.52, $J_{4,5}$ 5.06
16	4.38, $J_{1,2}$ 6.89	4.96	5.19, *J* 8.80	4.96	3.41, $J_{5,5'}$ 11.70, $J_{4,5'}$ 8.65	4.11, $J_{5,5'}$ 11.76, $J_{4,5}$ 4.75
17	4.37, $J_{1,2}$ 7.61	4.94	5.31, *J* 9.40	4.94	3.32, $J_{5,5'}$ 11.42, $J_{4,5'}$ 9.86	4.13, $J_{5,5'}$ 11.57, $J_{4,5}$ 5.54
18	4.44, $J_{1,2}$ 6.96	4.96	5.21, *J* 7.72	4.96	3.33, $J_{5,5'}$ 11.81, $J_{4,5'}$ 7.75	4.10, $J_{5,5'}$ 11.72, $J_{4,5}$ 5.06

Table 2
300 MHz ^{1}H NMR data ($CDCl_3$) for functional groups of *O*-acyl-β-D-xylopyranosides [a]

Compd	Chemical shifts (δ, ppm)						
	OPiv (s)			OAc (s)			OMe (s)
	2	3	4	2	3	4	
2	1.24						3.38
3		1.25					3.53
4			1.23				3.53
5	1.19	1.18					3.46
6	1.23		1.24				3.47
7		1.20	1.17				3.54
8	1.15	1.13	1.17				3.48
9				2.13	2.11		3.47
10				2.10		2.08	3.47
11				2.05	2.04	2.07	3.47
12	1.17				2.02	2.07	3.47
13		1.20		2.07			3.47
14		1.15		2.03		2.05	3.48
15			1.18	2.05	2.02		3.48
16	1.18	1.14				2.02	3.47
17	1.16		1.18		1.99		3.48
18		1.14	1.16	2.04			3.47

[a] All signals are singlets

measured using the Optical Activity AA-10 Automatic Polarimeter at ~ 20 °C using $CHCl_3$ as solvent.

Selective pivaloylations of methyl β-D-xylopyranoside (**1**).—(a) To a solution of **1** (500 mg, 3 mmol) in dry pyridine (7.5 mL) was added pivaloyl chloride (1.5 mL, 15 mmol). The mixture was stirred at ambient temperature for 48 h, and the reaction was stopped by the addition of 96% EtOH. Water was added, and the mixture of solvents evaporated under reduced pressure. The remaining traces of water were removed by co-distillation with toluene. Column chromatography of the residue (solvent A, 2:1) gave, first, crystalline methyl 2,3,4-*O*-pivaloyl-β-D-xylopyranoside (**8**; 792 mg, 63%); R_f ~ 0.90; mp 102–105 °C (from EtOAc–light petroleum); $[\alpha]_D$ −44° (*c* 1.0). Anal. Calcd for $C_{21}H_{36}O_8$: C, 60.58; H, 8.65. Found: C, 60.76; H, 8.88. Eluted next was crystalline methyl 2,4-di-*O*-pivaloyl-β-D-xylopyranoside (**6**; 65 mg, 7%); R_f ~ 0.70; mp 57–59 °C (from EtOAc–light petroleum); $[\alpha]_D$ −55° (*c* 1.0). Anal. Calcd for $C_{16}H_{28}O_7$: C, 57.83; H, 8.43. Found: C, 57.74; H, 8.52. Eluted last was crystalline methyl 3,4-di-*O*-pivaloyl-β-D-xylopyranoside (**7**; 187 mg, 19%); R_f ~ 0.55; mp 125–127 °C (from EtOAc–light petroleum); $[\alpha]_D$ −40° (*c* 1.0). Anal. Calcd for $C_{16}H_{28}O_7$: C, 57.83; H, 8.43. Found: C, 57.96; H, 8.42.

Conventional acetylation of **6** (100 mg, 0.3 mmol) with Ac_2O–pyridine, followed by column chromatography (solvent A, 5:1) and crystallization (EtOAc–light petroleum) gave the 3-acetate **17** (96 mg, 86%); R_f ~ 0.75; mp 87–89 °C; $[\alpha]_D$ −45° (*c* 1.0). Anal. Calcd for $C_{18}H_{30}O_8$: C 57.75; H, 8.02. Found: C, 57.89; H, 7.95. Likewise, **7** gave the 2-acetate **18** (110 mg, 96%); R_f ~ 0.67; mp 114–116 °C (from EtOAc–light petroleum); $[\alpha]_D$ −52° (*c* 1.0). Anal. Calcd for $C_{18}H_{30}O_8$: C, 57.75; H, 8.02. Found: C, 57.72; H, 7.97.

(b) Pivaloylation of **1** (200 mg, 1.2 mmol) with pivaloyl chloride (0.6 mL, 6 mmol) for 24 h at 60 °C, followed by column chromatography (solvent A, 2:1) of the product gave the 2,3,4-tripivalate **8** (460 mg, 90%).

(c) Pivaloylation of **1** (1 g, 6 mmol) with pivaloyl chloride (2 mL, 18 mmol) in dry pyridine (10 mL) for 2 h as described in (a), followed by column chromatography (solvent A, 2:1) of the product gave the 2,3,4-tripivalate **8** (180 mg, 7%), followed by **6** (511 mg, 25%) and **7** (536 mg, 26%). Eluted last was methyl 2,3-di-*O*-pivaloyl-β-D-xylopyranoside as a glass (**5**; 19 mg, 1%); R_f ~ 0.48; $[\alpha]_D$ −47° (*c* 1.0). Anal. Calcd for $C_{16}H_{28}O_7$: C, 57.83; H, 8.43. Found: C 57.89; H, 8.50.

Conventional acetylation of **5** (10 mg, 0.03 mmol) with Ac_2O–pyridine, followed by column chromatography (solvent A, 5:1) gave the 4-acetate **16** as a glass (9 mg, 86%); R_f ~ 0.64; $[\alpha]_D$ −50° (*c* 0.5). Anal. Calcd for $C_{18}H_{30}O_8$: C, 57.75; H, 8.02. Found: C, 57.62; H, 8.15.

(d) Pivaloylation of **1** (500 mg, 3 mmol) with pivaloyl chloride (0.35 mL, 3 mmol) in dry pyridine (2 mL) for 45 min as described in (a), followed by column chromatography (solvent A, 1:5) of the product gave the 2,3,4-tripivalate **8** (40 mg, 3%), a mixture of the dipivalates **5**, **6**, and **7** (85 mg 8%), followed by crystalline methyl 3-*O*-pivaloyl-β-D-xylopyranoside (**3**; 150 mg, 20%); R_f ~ 0.50; mp 117–119 °C (from EtOAc–light petroleum); $[\alpha]_D$ −25° (*c* 1.0). Anal. Calcd for $C_{11}H_{20}O_6$: C, 53.23; H, 8.06. Found: C, 53.06; H, 7.96. Eluted next was crystalline methyl 4-*O*-pivaloyl-β-D-xylopyranoside (**4**; 165 mg, 22%); R_f ~ 0.29; mp 113–115 °C; $[\alpha]_D$ −68° (*c* 1.0). Anal. Calcd for $C_{11}H_{20}O_6$: C, 53.23; H, 8.06. Found: C, 53.14; H, 8.28. A mixture of **4** and methyl 2-*O*-pivaloyl-β-D-xylopyranoside (**2**, R_f ~ 0.29) (15 mg, 2%) followed. Eluted last was some unreacted **1** (140 mg, 28%).

Conventional acetylation of **3** (100 mg, 0.4 mmol)

with Ac_2O–pyridine, followed by column chromatography (solvent A, 2:1) gave the 2,4-diacetate **14** (115 mg, 87%); $R_f \sim 0.66$; mp 114–116 °C (from EtOAc–light petroleum); $[\alpha]_D$ −48° (*c* 1.0). Anal. Calcd for $C_{15}H_{24}O_8$: C, 54.22; H, 7.23. Found: C, 54.45; H, 7.44. Likewise, **4** gave the 2,3-diacetate **15** (120 mg, 90%); $R_f \sim 0.73$; mp 105–107 °C (from di-isopropylether); $[\alpha]_D$ −57° (*c* 1.0). Anal. Calcd for $C_{15}H_{24}O_8$: C, 54.22; H, 7.23. Found: C, 54.21; H, 7.29. A mixture of **4** and **2** (12 mg, 0.05 mmol) was also acetylated and chromatographed (solvent A, 2:1) to give a mixture of **15** and the 3,4-diacetate **12** ($R_f \sim 0.71$) (10 mg, 62%).

Conventional acetylation of **1** (500 mg, 3 mmol) with Ac_2O–pyridine gave the triacetate **11** (869 mg, 97%); R_f 0.75 (solvent A, 1:2); mp 116–118 °C (from EtOAc–light petroleum), lit. 115 °C [21]; $[\alpha]_D$ −65° (*c* 2.7), lit −60.8° [21].

Enzymic deacylations.—Rabbit serum and a purified esterase fraction from rabbit serum were prepared as previously described [13]. A solution of the substrate in phosphate-buffered saline (PBS, 0.01 M) or a mixture of PBS and Me_2SO was incubated with rabbit serum or the esterase at 37 °C. The pH was maintained at 7.2 by the addition of 0.1 M NaOH, and each reaction was monitored by TLC. Reactions were stopped by adding EtOH, the precipitated proteins were removed by centrifugation, the solvents evaporated under reduced pressure, and the product subjected to column chromatography. Control reactions were performed parallel to every enzymic hydrolysis. Controls contained all reactants except the enzyme.

(a) Incubation of the 3,4-dipivalate **7** (100 mg, 0.3 mmol) in Me_2SO (2 mL) and PBS (10 mL) with rabbit serum (1.5 mL) for 24 h, followed by column chromatography (solvent A, 1:5) gave the 3-pivalate **3** (25 mg, 33%) and the 4-pivalate **4** (31 mg, 41%).

Hydrolysis of **7**, as described above, with the purified esterase (500 μL) for 24 h, yielded **3** (33 mg, 44%) and **4** (42 mg, 32%).

(b) Incubation of the 2,4-dipivalate **6** (100 mg, 0.3 mmol) with rabbit serum (2 mL), for 48 h as described in (a), followed by column chromatography (solvent A, 2:1) yielded the starting compound **6** (30 mg, 30%), the 3,4-dipivalate **7** (24 mg, 24%), and a mixture of the 3-pivalate **3** and the 4-pivalate **4** (14 mg, 19%).

Hydrolysis of **6**, as described above, with the purified esterase (1 mL) for 96 h, followed by chromatography, produced **6** (35 mg, 35%), **7** (22 mg, 22%), and a mixture of **3** and **4** (18 mg, 24%).

Parallel control incubations were monitored by TLC (solvent A, 2:1) showing the formation of **7**, but not **3** and **4**.

(c) Incubation of the 2,3,4-triacetate **11** (100 mg, 0.35 mmol) in PBS (10 mL) with rabbit serum (1 mL) for 4 h, followed by chromatography (solvent A, 1:2) gave methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside **10** as a glass (50 mg, 58%); $R_f \sim 0.44$; $[\alpha]_D$ −59° (*c* 1.0). Anal. Calcd for $C_{11}H_{16}O_7$: C, 48.39; H, 6.45. Found: C, 48.52; H, 6.39. Some unreacted **11** (30 mg, 30%) was recovered as well.

Hydrolysis of **11** (200 mg, 0.7 mmol), as described above, with the purified esterase (300 μL) gave **10** (92 mg, 53%) and **11** (65 mg, 33%).

Pivaloylation of **10** (50 mg, 0.2 mmol) with pivaloyl chloride (100 mL) in dry pyridine (1 mL) for 24 h at ambient temperature, followed by column chromatography (solvent A, 2:1) gave methyl 2,4-di-*O*-acetyl-3-*O*-pivaloyl-β-D-xylopyranoside (**14**; 63 mg, 95%).

(d) Incubation of **14** (50 mg, 0.15 mmol) in Me_2SO (1 mL) and PBS (5 mL) with rabbit serum (1 mL) for 5 h, followed by chromatography (solvent A, 1:2) produced 2,4-diacetate **10** (20 mg, 54%) and the starting **14** (10 mg, 20%).

Hydrolysis of **14**, as described above, with the purified esterase (300 μL) gave **10** (24 mg, 64%) and **14** (8 mg, 16%).

(e) Incubation of **15** (50 mg, 0.15 mmol) in Me_2SO (1 mL) and PBS (5 mL) with rabbit serum (1.5 mL) for 72 h, followed by chromatography (solvent A, 1:2) produced 2,3-diacetate **9** (6 mg, 16%), $R_f \sim 0.58$, and the starting **15** (26 mg, 52%).

Hydrolysis of **15**, as described above, with the purified esterase (500 μL) for 48 h gave **9** (10 mg, 27%) and **15** (21 mg, 42%).

(f) Incubation of **18** (100 mg, 0.3 mmol) in Me_2SO (2 mL) and PBS (10 mL) with rabbit serum (1.5 mL) for 28 h, followed by chromatography (solvent A, 2:1) produced methyl 2-*O*-acetyl-3-*O*-pivaloyl-β-D-xylopyranoside (**13**; 48 mg, 55%); $R_f \sim 0.30$; mp 88–90 °C; $[\alpha]_D$ −22° (*c* 0.5). Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.79; H, 7.59. Found: C, 53.83; H, 7.83. Some unreacted **18** (25 mg, 25%) was recovered as well.

Hydrolysis of **18**, as described above, with the purified esterase (500 μL) gave **13** (50 mg, 57%) and **18** (25 mg, 25%).

Conventional acetylation of **13** (20 mg, 0.07 mmol) with Ac_2O-pyridine, followed by chromatography (solvent A, 2:1) gave the 2,4-diacetate **14** (21 mg, 92%).

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