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Syntheses of partially pivaloylated D-glucopyranoses: new substrates for the esterase from rabbit serum

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ABSTRACT

The selective pivaloylation (pivaloyl chloride–pyridine) of D-glucopyranose proceeds by two pathways after initial 6-pivaloylation, namely, successive esterification of (*a*) positions 1, 3, 4, and 2 (major pathway) and (*b*) positions 2, 1, 4, and 3 (minor pathway). Numerous di- (**3** and **4**), tri- (**5–9**), and tetra-pivalates (**10–12**) have been prepared and characterised. On treatment of the 2,6-dipivalate (**4**) with rabbit serum or partially purified esterase II, PivO-6 was hydrolysed selectively, whereas the 1,6-dipivalate (**3**) underwent partial 1 → 2 acyl migration to give **4** and enzymic de-esterification of **3** and **4** occurred simultaneously.

INTRODUCTION

The regioselective acylation of sugars can be carried out rarely with efficiency[1,2]. The ability of pivaloyl chloride to acylate sugars selectively stimulated syntheses of partially pivaloylated derivatives of simple glycosides[3–5] and disaccharides[6,7]. Pivaloyl derivatives of methyl α-D-glucopyranoside are good substrates for esterases from mammalian sera[8–10] and are hydrolysed regioselectively to give compounds with unsubstituted primary hydroxyl groups. ^{14}C-Labelled methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside has been used[10] to monitor the isolation of esterase from rabbit serum.

Regioselective enzymic acylations[11–19] and deacylations[20–23] in the carbohydrate field have been developed only recently. Numerous furanose and pyranose derivatives[11,13], di- and oligo-saccharides, and other sugar-containing compounds[10,14] have been selectively acylated by lipases[11,13,18,19] or proteolytic enzymes[12]. Also, enzyme-catalysed trans-esterifications between secondary hy-

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droxyl groups and an activated ester (usually a trihaloethyl carboxylate) have been observed[15–17].

Lipase-catalysed regioselective cleavage of the 1-*O*-acyl groups of acylated carbohydrates[20], selective hydrolyses of the primary acyl groups of methyl 2,3,4,6-tetra-*O*-acylhexopyranosides[13,21], and deacylations of 3′,5′-di-*O*-acylpyrimidine nucleosides by subtilisin[22] have been reported.

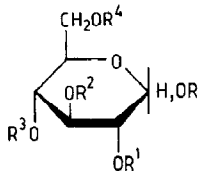
We now report the synthesis of a series of partially pivaloylated derivatives of D-glucopyranose and their use as substrates for the esterase from rabbit serum.

RESULTS AND DISCUSSION

Unless noted otherwise, the following pivaloylations were conducted in dry pyridine at room temperature.

Treatment of D-glucopyranose (**1**) with 5 equiv of pivaloyl chloride for 24 h gave 18% of the 1,2,3,4,6-pentapivalate (**13**), 16% of a 1:1 mixture of the 1,2,4,6- (**12**) and 1,3,4,6-tetra-pivalate (**11**), and 15% of the 1,2,3,6-tetrapivalate (**10**). Attempts to resolve the mixture of **11** and **12** failed. However, **11** and **12** could be synthesised by treatment of the 1,3,6- (**8**) and 1,2,6-tripivalate (**5**), respectively, with 10 equiv of pivaloyl chloride. Thus, **5** gave, after chromatography, **10** and **12** in the ratio 1:2, and **8** gave **10**, **11**, and **13** in the ratios 1:3.2:1. The crystalline tetrapivalates **11** and **12** were obtained and characterised as the acetates **24** and **25**, respectively.

Treatment of **1** with 10 equiv of pivaloyl chloride at 60° for 3 days gave only 50%



1	R = R^1 = R^2 = R^3 = R^4 = H	**14**	R = Piv, R^1 = R^2 = R^3 = R^4 = Ac
2	R = R^1 = R^2 = R^3 = H, R^4 = Piv	**15**	R = R^1 = R^2 = R^3 = Ac, R^4 = Piv
3	R = R^4 = Piv, R^1 = R^2 = R^3 = H	**16**	R = R^4 = Piv, R^1 = R^2 = R^3 = Ac
4	R = R^2 = R^3 = H, R^1 = R^4 = Piv	**17**	R = R^2 = R^3 = Ac, R^1 = R^4 = Piv
5	R = R^1 = R^4 = Piv, R^2 = R^3 = H	**18**	R = R^1 = R^4 = Piv, R^2 = R^3 = Ac
6	R = R^3 = R^4 = Piv, R^1 = R^2 = H	**19**	R = R^3 = R^4 = Piv, R^1 = R^2 = Ac
7	R = R^3 = H, R^1 = R^2 = R^4 = Piv	**20**	R = R^3 = Ac, R^1 = R^2 = R^4 = Piv
8	R = R^2 = R^4 = Piv, R^1 = R^3 = H	**21**	R = R^2 = R^4 = Piv, R^1 = R^3 = Ac
9	R = R^2 = H, R^1 = R^3 = R^4 = Piv	**22**	R = R^2 = Ac, R^1 = R^3 = R^4 = Piv
10	R = R^1 = R^2 = R^4 = Piv, R^3 = H	**23**	R = R^1 = R^2 = R^4 = Piv, R^3 = Ac
11	R = R^2 = R^3 = R^4 = Piv, R^1 = H	**24**	R = R^2 = R^3 = R^4 = Piv, R^1 = Ac
12	R = R^1 = R^3 = R^4 = Piv, R^2 = H	**25**	R = R^1 = R^3 = R^4 = Piv, R^2 = Ac
13	R = R^1 = R^2 = R^3 = R^4 = Piv	**26**	R = R^2 = R^3 = R^4 = Ac, R^1 = Piv

TABLE I

Analytical data for compounds **2–26**

Compound	Mp [a] (degrees)	$[\alpha]_D^{22}$ [b] (degrees)	Formula	Anal.			
				Calcd		Found	
				C	H	C	H
2	161–163	−69 (MeOH)	$C_{11}H_{20}O_7$	50.02	7.58	50.10	7.59
3	glass	−13	$C_{16}H_{28}O_8$	55.16	8.10	54.96	7.99
4	151–153	+51				55.34	7.98
5	156–157	+29	$C_{21}H_{36}O_9$	58.31	8.39	58.56	8.10
6	129–131	−4				58.47	8.21
7	123–125	+37				58.12	8.70
8	135–136	−22				58.36	8.09
10	144–145	+21	$C_{26}H_{44}O_{10}$	60.62	8.55	60.55	8.25
11	168–170	+15				60.52	8.39
12	172–174	−4				60.62	8.45
13 [c]	156–157	+11	$C_{31}H_{52}O_{11}$	61.98	8.73	61.78	8.63
14	141–143	−2	$C_{19}H_{28}O_{11}$	52.77	6.53	52.60	6.75
15	105–107	+92				52.71	6.74
16	180–181	+22	$C_{22}H_{34}O_{11}$	55.69	7.22	55.50	7.06
17	193–195	+12				55.67	7.18
18	162–164	+14	$C_{25}H_{40}O_{11}$	58.12	7.81	58.27	7.75
19	178–180	+8				58.24	7.68
20	159–161	+78				58.22	7.61
21	174–175	+22				58.22	7.75
22	126–128	+98				57.97	7.59
23 [d]	140–141	+20	$C_{36}H_{58}O_{17}$	56.68	7.66	56.68	7.50
24	202–204	+8	$C_{28}H_{46}O_{11}$	60.20	8.30	60.40	8.39
25	225–227	+11				60.43	8.45
26	172–173	+9	$C_{19}H_{28}O_{11}$	52.77	6.53	52.57	6.76

[a] From isopropyl ether–light petroleum. [b] In chloroform, if not stated otherwise. [c] Lit.[24] mp 156–157° (from EtOH), $[\alpha]_D$ +10.9°. [d] Analytical data for $C_{28}H_{46}O_{11}\cdot 2Ac_2O$.

of the pentapivalate **13** (cf. the reaction[24] of **1** with pivaloyl chloride in pyridine–chloroform at 75–80° for 5 days, which gave 74% of **13**). These results illustrate the steric effects associated with the bulky pivaloyl group. The β configurations of **10–13** were confirmed by the ^{1}H-NMR data.

With 3 equiv of pivaloyl chloride, **1** gave at least nine products and chromatography of the mixture yielded the 1,3,6- (**8**, 48%), 1,4,6- (**6**, 9%), and 1,2,6-tripivalate (**5**, 9%), and the 1,6-dipivalate (**3**, 28%), and they were characterised as the acetylated derivatives **21**, **19**, **18**, and **16**, respectively (Table I). The structures of these compounds were confirmed by analytical and ^{1}H-NMR data (Table II).

Treatment of **1** with 2 equiv of pivaloyl chloride gave the 2,6-di- (**4**) and 1,6-di-pivalate (**3**), and the 6-pivalate (**2**) in the ratios 1:7:2.7. The signal for H-1 (δ 5.66, $J_{1,2}$ 7.32 Hz) in the ^{1}H-NMR spectrum of **3** indicated that HO-1 was substituted, and the corresponding data for **4** and **2** indicated that HO-1 was unsubstituted. The favoured pivaloylation of HO-1 accords with its higher acidity[1].

TABLE II

^{1}H-NMR data ($CDCl_3$), 100 MHz for pivaloyl derivatives of D-glucopyranose

Compd	Chemical shifts (ppm)										
	H-1(d, $J_{1,2}$ Hz)	Me_3C–CO [a]					AcO [a]				
		1	2	3	4	6	1	2	3	4	6
3	5.66 (7.32)	1.19				1.22					
4			1.23 [b]			1.22 [b]					
5	5.64 (8.20)	1.19	1.18			1.21					
6	5.51 (7.91)	1.19			1.24 [b]	1.23 [b]					
7			1.19	1.24 [b]		1.20 [b]					
8	5.59 (7.91)	1.19		1.24 [b]		1.21 [b]					
9			1.22 [b]		1.23 [b]	1.22 [b]					
10	5.69 (8.20)	1.19	1.13	1.17		1.22					
11	5.61 (8.20)	1.19		1.17	1.16	1.20					
12	5.66 (8.20)	1.19	1.20 [b]		1.22 [b]	1.20 [b]					
13	5.71 (8.20)	1.18	1.12	1.12	1.15	1.21					
14	5.67 (7.91)	1.20						2.01	2.01	2.03	2.09
15	6.32 (3.81)					1.21	2.17	2.03	2.03	2.04	
16	5.66 (7.91)	1.19				1.21		2.01	2.01	2.03	
17	5.74 (7.62)		1.14			1.22	2.08		1.99	2.03	
18	5.69 (8.20)	1.18	1.13			1.21			1.99	2.03	
19	5.66 (7.91)	1.19			1.16	1.21		2.01	1.99		
20	6.36 (3.81)		1.13	1.13		1.21	2.15			2.02	
21	5.66 (8.20)	1.18		1.13		1.21		1.99		2.01	
22	6.37 (3.52)		1.13		1.15	1.22	2.17		1.98		
23	5.71 (7.91)	1.18	1.12	1.12		1.21				2.01	
24	5.66 (8.20)	1.18		1.12	1.15	1.21		1.98			
25	5.70 (7.91)	1.18	1.12		1.16	1.21			1.96		
26	5.76 (7.32)		1.14				2.09		1.99	2.03	2.09

[a] All signals are singlets. [b] Assignments could be reversed.

Compounds **2–4** were characterised as the acetylated derivatives **15–17**, respectively.

A small yield of the 6-pivalate (**2**) was isolated after treatment of **1** with 1 equiv of pivaloyl chloride. The ^{1}H-NMR spectrum (CD_3OD) of **2** contained one sharp signal (s, δ 1.22) that was attributed to PivO-6, but traces of other monopivalates could not be excluded. Acetylation of **2** gave an α,β-mixture of the tetra-acetates from which the crystalline α anomer (**15**α) was isolated.

In order to clarify the pattern of reaction of **1** with pivaloyl chloride, the 1,6- (**3**) and 2,6-dipivalate (**4**) were each treated with 1 equiv of pivaloyl chloride. Thus, **3** gave, after chromatography, the 1,3,6-tripivalate (**8**, 52%) together with small proportions of the 1,2,6- (**5**) and 1,4,6-tripivalate (**6**). The ^{1}H-NMR spectrum of **8** contained, inter alia, a signal at δ 5.02 (t, $J_{2,3}$ 9.08, $J_{3,4}$ 8.50 Hz) that indicated HO-3 to be substituted. Pivaloylation of HO-3 in **3** rather than HO-2 may reflect steric hindrance, but also accords with the reported higher reactivity of HO-3 in the *β-gluco* series. The formation of **5** and **6** in equal proportions indicates that HO-2 and HO-4 have similar reactivities.

Pivaloylation of the 2,6-dipivalate (**4**) afforded the 2,4,6- (**9**), 2,3,6- (**7**), and 1,2,6-tripivalate (**5**) in the ratios 1:1.3:5.2, which were characterised as the acetylated derivatives **22**, **20**, and **18**, respectively.

The foregoing results indicate that the pivaloylation of D-glucopyranose (**1**) yields first the 6-pivalate (**2**), which is followed in the major pathway by successive pivaloylation at HO-1, HO-3, HO-4, and HO-2, and, in the minor pathway, at HO-2, HO-1, HO-4, and HO-3.

The partially pivaloylated D-glucoses **2–13** and the respective acetylated derivatives **14–26** (Table I) can be differentiated readily on the basis of their chromatographic mobilities and ^{1}H-NMR data (Table II).

The susceptibility of the 1,6- (**3**) and 2,6-dipivalate (**4**) to rabbit serum and esterase II[10] isolated therefrom was examined. Since the resulting monopivalates had identical mobilities in TLC and no reference compounds were available, they were characterised as the tetra-acetates. In this manner, the tetra-acetate (**26**) of the 2-pivalate was the only product isolated (76%) after the action of rabbit serum on **4**, whereas, after the action of esterase II, 85% of a 7:1 mixture of the acetylated 2- (**26**) and 6-pivalate (**15**) was obtained from which **26** was isolated by crystallisation. The relative resistance of PivO-2 in **4** to enzymic hydrolysis is in agreement[8,9] with the pattern of stepwise de-esterification of methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside by esterases from different sources. The regioselective removal of PivO-6 was also observed[10] in the enzymic hydrolysis of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranoside.

Rabbit serum catalysed the hydrolysis of the 1,6-dipivalate (**3**) to give, after acetylation, 42% of a 1:3.4 mixture of the acetylated 6- (**15**) and 2-pivalate (**26**). Esterase II reacted with **3** to give, after acetylation, mainly **26** (37%). The formation of the 2-pivalate in the enzymic deacylation of the 1,6-dipivalate (**3**) can be explained only by acyl migration. When **3** was incubated in phosphate-buffered

saline in the absence of esterase, a product was formed which co-migrated in TLC with the 2,6-dipivalate (**4**), and an equilibrium of **3** and **4** was established with a 40% decrease in the intensities of the signals for H-1 (d, δ 5.66) and PivO-1 (s, δ 1.19) with corresponding increases in that (s, δ 1.22) due to PivO-2,6 (see Table II). The migration of PivO-1 was confirmed by the isolation of the 2,3,4- (**16**) and 1,3,4-triacetate (**17**) after acetylation.

Hydrolysis of PivO-1 in the 1,6-dipivalate (**3**) was faster than that of PivO-6, as indicated by the absence of a resonance for PivO-1 in the ^{1}H-NMR spectrum of the products of enzymic hydrolysis. Acyl migration also occurred when the pH was lowered to 6.3 and in pure water (data not presented). Some 2 → 1 acyl migration was also observed during the hydrolysis of the 2,6-dipivalate (**4**). Due to the faster hydrolysis of PivO-1 and PivO-6 than of PivO-2, only the 2-pivalate was isolated.

Many examples of acyl migration in the *gluco* series have been reported[1]. Generally, the migration occurs from O-1 towards O-6 with 1 → 2 migration observed most often as in **3**. In contrast to the above results, methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside and methyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α-D-glucopyranoside did not undergo acyl migrations[8,10]. However, 2 → 4 acyl migration in methyl 2,6-di-*O*-pivaloyl-α-D-glucopyranoside was observed[9] in transformations caused by sera of ruminants, but the enzyme was not identified.

EXPERIMENTAL

General methods.—Melting points are uncorrected. Optical rotations were determined for 1% solutions in chloroform if not stated otherwise. Column chromatography was performed on silica gel (Merck) and TLC on Kieselgel G (Merck) with *A*, benzene–ethyl acetate (in the proportions given); *B*, ether–light petroleum (1 : 1); and *C*, acetonitrile–water (5 : 1); and detection by charring with H_2SO_4. The physical constants and elemental analyses of **2–8** and **10–26** are given in Table I. The ^{1}H-NMR spectra (100 MHz, $CDCl_3$, internal Me_4Si) were recorded with a Jeol JNM FX-100 FT spectrometer, and the data for **3–26** are given in Table II.

Selective pivaloylation of D-*glucopyranose* (**1**).—(*a*) To a solution of **1** (180 mg, 1 mmol) in dry pyridine (2 mL) was added pivaloyl chloride (625 μL, 5 mmol). The mixture was stirred at ambient temperature for 24 h, EtOH (2 mL) was added, the mixture was concentrated, and traces of pyridine were removed by evaporation of toluene from the residue. Column chromatography (solvent *A*, 10 : 1) gave, first, 1,2,3,4,6-penta-*O*-pivaloyl-β-D-glucopyranose (**13**; 134 mg, 26%; R_F ~ 0.85). Eluted next was a 1 : 1 mixture (193 mg, 45%; R_F ~ 0.43) that was shown by TLC (solvent *B*) to comprise 1,2,4,6- (**12**, R_F ~ 0.67) and 1,3,4,6-tetra-*O*-pivaloyl-β-D-glucopyranose (**11**, R_F ~ 0.57). Eluted last was 1,2,3,6-tetra-*O*-pivaloyl-β-D-glucopyranose (**10**; 89 mg, 21%; R_F ~ 0.29).

Conventional treatment of **10** with acetic anhydride–pyridine for 16 h at ambient temperature afforded, after column chromatography (solvent *A*, 10 : 1), the 4-acetate **23** (51 mg, 95%).

(*b*) Pivaloylation of **1** (180 mg) with 3 equiv of pivaloyl chloride (375 μL) in dry pyridine (2 mL), as in (*a*), followed by column chromatography (solvent *A*, 1:1) of the product, gave the 1,3,6- (**8**; 208 mg, 48%; $R_F \sim 0.83$), 1,4,6- (**6**; 39 mg, 9%; $R_F \sim 0.67$), and 1,2,6-tripivalate (**5**; 40 mg, 9%; $R_F \sim 0.55$). Elution with solvent *C* then afforded 1,6-di-*O*-pivaloyl-β-D-glucopyranose (**3**; 98 mg, 28%).

Conventional acetylation of **3** (50 mg), followed by column chromatography (solvent *A*, 2:1) and crystallisation, gave the 2,3,4-triacetate **16** (64 mg, 94%). Likewise, **5** gave the 3,4-diacetate **18** (57 mg, 96%), **6** gave the 2,3-diacetate **19** (61 mg, 98%), and **8** gave the 2,4-diacetate **21** (55 mg, 92%).

(*c*) Reaction of **1** (180 mg) with 2 equiv of pivaloyl chloride (250 μL) in dry pyridine (2 mL), as in (*a*), gave, after column chromatography (solvent *C*), the 2,6- (**4**; 15 mg, 3%; $R_F \sim 0.91$) and 1,6-dipivalate (**3**; 54 mg, 21%; $R_F \sim 0.84$), and the 6-pivalate (**2**; 22 mg, 8%; $R_F \sim 0.70$).

Acetylation of **2** (50 mg), followed by column chromatography (solvent *A*, 2:1), gave the 2,3,4,6-tetra-acetate **15** (66 mg, 98%). Likewise, **4** gave the 1,3,4-triacetate **17** (65 mg, 96%).

Other pivaloylations. — *(a) 1,6-Di-O-pivaloyl-β-D-glucopyranose* (**3**). To a solution of **3** (323 mg, 0.93 mmol) in dry pyridine (6.6 mL) was added pivaloyl chloride (116 μL, 0.93 mmol). The mixture was stored for 24 h at ambient temperature, then diluted with EtOH (2 mL), and concentrated. Column chromatography (solvent *A*, 2:1) of the residue gave the 1,3,6- (**8**; 225 mg, 52%; $R_F \sim 0.68$), 1,4,6- (**6**; 30 mg, 8%; $R_F \sim 0.49$), and 1,2,6-tripivalate (**5**; 30 mg, 8%; $R_F \sim 0.38$).

(*b*) *2,6-Di-O-pivaloyl-D-glucopyranose* (**4**). To a solution of **4** (350 mg, 1 mmol) in dry pyridine (2 mL) was added pivaloyl chloride (125 μL, 1 mmol). The mixture was stirred for 16 h at ambient temperature, then worked-up as described for **3**. Column chromatography (solvent *A*, 2:1) of the product gave a mixture of the tetrapivalate (54 mg, 10%), 2,4,6- (**9**; 27 mg, 6%; $R_F \sim 0.77$), 2,3,6- (**7**; 35 mg, 8%; $R_F \sim 0.62$), and 1,2,6-tripivalate (**5**; 132 mg, 31%; $R_F \sim 0.38$), and **4** (60 mg, 17%; $R_F \sim 0.28$).

Conventional acetylation of **7** (50 mg) gave the 1,4-diacetate (**20**; 56 mg, 95%). Likewise, **9** (50 mg) gave the 1,3-diacetate (**22**; 57 mg, 96%).

(*c*) *1,2,6-Tri-O-pivaloyl-β-D-glucopyranose* (**5**). Treatment of **5** (100 mg, 0.23 mmol) with pivaloyl chloride (290 μL, 2.3 mmol) in dry pyridine (2 mL) for 16 h at ambient temperature, followed by column chromatography (solvent *A*, 10:1), gave the 1,2,4,6- (**12**; 61 mg, 59%) and 1,2,3,6-tetrapivalate (**10**; 36 mg, 30%).

Conventional acetylation of **12** (50 mg) gave the 3-acetate (**25**; 53 mg, 97%).

(*d*) *1,3,6-Tri-O-pivaloyl-β-D-glucopyranose* (**8**). Treatment of **8** (172 mg, 0.4 mmol) with pivaloyl chloride (500 μL, 4 mmol) in dry pyridine (2 mL) for 16 h at ambient temperature, followed by column chromatography (solvent *A*, 10:1), afforded the 1,2,3,4,6-pentapivalate **13** (35 mg, 15%), and the 1,3,4,6- (**11**; 99 mg, 48%) and 1,2,3,6-tetrapivalate (**10**; 31 mg, 15%).

Conventional acetylation of **11** (50 mg) gave the 2-acetate (**24**; 52 mg, 96%).

2,3,4,6-Tetra-O-acetyl-1-O-pivaloyl-β-D-glucopyranose (**14**).—To a solution of

2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (174 mg, 0.5 mmol) in dry pyridine (1 mL) was added pivaloyl chloride (125 μL), and the mixture was stirred for 16 h at ambient temperature, then concentrated. Column chromatography (solvent *A*, 1:1) of the residue gave **14** (140 mg, 65%).

Enzymic deacylations.—Rabbit serum and esterase II were prepared as described[10]. A solution of the substrate in phosphate-buffered saline (PBS, 0.01 M, 10 mL) was incubated with rabbit serum or esterase II at 37°. The pH was maintained at 7.2 by the addition of 0.1 M NaOH and each reaction was monitored by TLC (solvent *C*). Reaction was stopped by adding EtOH (10 mL), the precipitated proteins were removed by centrifugation, the solvent was evaporated, the residue was acetylated conventionally, and the product was subjected to column chromatography (solvent *A*, 5:1).

(*a*) Treatment of the 2,6-dipivalate **4** (100 mg) with rabbit serum (2 mL) for 15 h, followed by acetylation and column chromatography, as described above, gave the acetylated 2-pivalate **26** (94 mg, 76%) and traces of the acetylated 6-pivalate **15**.

Hydrolysis of **4** (100 mg) with esterase II (3.6 mg) for 3 h, followed by acetylation and column chromatography, afforded a mixture (100 mg, 85%) of **26** and **15** in the ratio 7:1 (^{1}H-NMR data). Crystallisation of the mixture from isopropyl ether–light petroleum gave **26** as needles.

(*b*) Treatment of the 1,6-dipivalate **3** (100 mg) with rabbit serum (2 mL) for 14 h, followed by acetylation and column chromatography (solvent *A*, 5:1), gave a mixture (47 mg, 42%) of the acetylated 6- (**15**) and 2-pivalate (**26**) in the ratio 1:3.4 (^{1}H-NMR data).

Hydrolysis of **3** (50 mg) with esterase II for 27 h, followed by acetylation and column chromatography (solvent *A*, 5:1), afforded a mixture (23 mg, 37%) of the acetylated 2- (**26**) and 6-pivalate (**15**) in the ratio 10:1. Crystallisation of the mixture from isopropyl ether–light petroleum gave **26**.

(*c*) Incubation of **3** (50 mg) in PBS (10 mL) at 37° for 14 h gave a 3:2 mixture of **3** and **4**. ^{1}H-NMR data: δ 5.66 (d, 0.56 H, $J_{1,2}$ 7.32 Hz, H-1), 1.22 (s, 15.57 H, PivO-2,6), 1.19 (s, 14.13 H, PivO-1).

Conventional acetylation of the above mixture, followed by column chromatography (solvent *A*, 5:1), gave acetylated 1,6- (**16**) and 2,6-dipivalate (**17**).

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