Carbohydrate Research, 252 (1994) 55–68
Elsevier Science B.V., Amsterdam

Towards the selective acylation of secondary hydroxyl groups of carbohydrates using oxime esters in an enzyme-catalyzed process

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(Received March 17 1993; accepted in revised form July 6th, 1993)

ABSTRACT

A lipase from *Candida antarctica* was used for the regioselective acylation and alkoxycarbonylation of the primary hydroxyl group of 2-deoxy-D-*arabino*-hexopyranose (**1**) and 2-deoxy-D-*lyxo*-hexopyranose (**2**) using oxime esters as acylating agents. Both, pyridine and 1,4-dioxane were used as solvents in the acylation process, but only 1,4-dioxane was effective for the alkyloxycarbonylation. A lipase from *Pseudomonas cepacia* catalyzed also the regioselective acylation of the primary hydroxyl group of **1** and **2** when 1,4-dioxane was used as solvent. Moreover, this lipase was a suitable biocatalyst for the acylation of the secondary HO-3 of 6-*O*(benzyloxy)carbonyl derivatives of 2-deoxy-D-hexoses. Subsequent deprotection of the primary hydroxyl group through catalytic hydrogenation readily afforded the 3-*O*-acyl derivatives of **1** and **2**. A study of the regioselective acylation and alkyloxycarbonylation of 1,6-anhydro-β-D-glucopyranose by the aforementioned lipase is also reported.

INTRODUCTION

Chemical transformations of sugars frequently involve acylation reations in protection–deprotection steps of their hydroxyl groups[1].

The enzymatic regioselective acylation of sugars is now well established; lipases[2] and proteases[3] have been applied successfully to the selective esterification[2,3] and hydrolysis[4] of free carbohydrates and their peracylated derivatives, respectively. Wong et al.[5] have reviewed recently the most important methods for the regioselective acylation of carbohydrates at the primary hydroxyl group. They emphasized that displacement of the equilibrium between acylation-hydrolysis towards the acylation requires, as well as an organic solvent, a large excess of the acylating agent, and such activated acyl donors as 2,2,2-trihaloethyl esters or enol esters. (The enol esters result in irreversible reactions, due to isomerization of the

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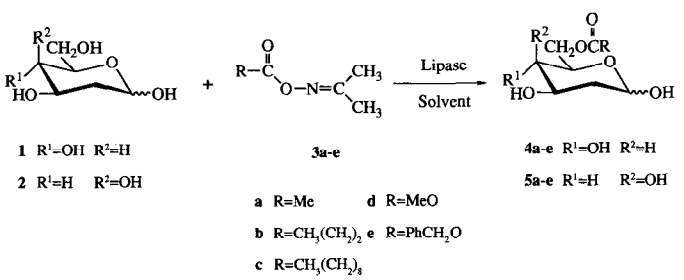
SSDI 0008-6215(93)E0241-R

released enol to the unreactive ketone or aldehyde, avoiding in the meantime product inhibition[6].) When acids act as acyl donors, high temperatures and diminished pressure must be used in order to shift the equilibrium towards the acylation reaction[7]. We have earlier reported an alternative procedure for the regioselective acylation of carbohydrates using oxime esters[8]. The good leaving group in these imino compounds favors the acylation process. Much current attention is focused on the relationship between the structure of the substrate and the activity of the enzyme[9]. Carbohydrates are excellent models for this study, since they contain multiple hydroxyl groups with different reactivities[10–14]. As a part of our program devoted to extend the enzyme-catalyzed regioselective acylation of carbohydrates with oxime esters, we report here the acylation of 2-deoxy-D-*arabino*-hexopyranose (**1**), of 2-deoxy-D-*lyxo*-hexopyranose (**2**), and of the conformationally rigid 1,6-anhydro-β-D-glucopyranose (**11**). Our interest in this work is to check how the absence of a hydroxyl group at C-2, and the rigid conformation, can affect the selectivity of the lipases tested.

RESULTS AND DISCUSSION

Regioselective acylation of 2-deoxy-D-hexoses.—2-Deoxy-D-*arabino*-hexopyranose (**1**) and 2-deoxy-D-*lyxo*-hexopyranose (**2**) react with oxime esters **3a–c**, in a 1:1 ratio at room temperature and dry pyridine as solvent under catalysis by *Candida antarctica* lipase to yield the corresponding 6-*O*-acylated derivatives **4a–c** and **5a–c**, respectively (Scheme 1 and Table I).

When 1,4-dioxane was used as solvent at room temperature, the yields decreased dramatically because of the lower solubility of the substrates. Nevertheless, careful control of the reaction time and use of a higher temperature (60°C) and a great excess of the acylating agent led to improved yields without affecting the selectivity (Table I). The modification at C-2 scarcely affected the yields with respect to those for the parent hexoses[8]. The position of acylation was determined by ^{13}C NMR spectroscopy (Table IV), taking into account the downfield shifts[15] of ~ 2.5 ppm for C-6 and the upfield shift of ~ 2.4 ppm for C-5, with respect to the δ values in the starting materials[16].



1 R^1=OH R^2=H
2 R^1=H R^2=OH

3a-e

4a-e R^1=OH R^2=H
5a-e R^1=H R^2=OH

a R=Me **d** R=MeO
b R=$CH_3(CH_2)_2$ **e** R=$PhCH_2O$
c R=$CH_3(CH_2)_8$

Scheme 1. Reaction of 2-deoxy-D-*arabino*-hexopyranose (**1**) and 2-deoxy-D-*lyxo*-hexopyranose (**2**) with acetone oxime esters **3a–c** and acetone *O*-[(alkoxy)carbonyl]oximes **3d, e**.

TABLE I

Reaction of 2-deoxy-D-*arabino*-hexopyranose (**1**) and 2-deoxy-D-lyxo-hexopyranose (**2**) with acetone oxime esters **3a–c**, and acetone *O*-[(alkoxy)carbonyl]oximes **3d** and **e**

Compd.	Lipase [a]	Solvent	*t* (°C)	Molar ratio [b]	t (h)	Yield (%) [c]	$[\alpha]_D^{25}$ (*c*, solvent)	R_f (solvent) [d]
4a	SP382	Pyridine	30	1:1	48	61	+53.4 (5.0, MeOH)	0.23 (*A*)
	SP382	1,4-Dioxane	60	1:3	72	54		
	SP435	Pyridine	30	1:1	48	67		
	SP435A	Pyridine	30	1:1	48	65		
	PS	Pyridine	30	1:2	48	32 [e]		
	PS	1,4-Dioxane	60	1:3	96	52		
4b	SP382	Pyridine	30	1:1	48	73	+58.2 (1.4, MeOH)	0.41 (*A*)
	SP382	1,4-Dioxane	60	1:3	72	71		
	PS	Pyridine	30	1:2	48	34 [f]		
	PS	1,4-Dioxane	60	1:3	96	50		
4c	SP382	Pyridine	30	1:1	48	78	+54.3 (1.1, MeOH)	0.18 (*B*)
	SP382	1,4-Dioxane	60	1:3	72	69		
	PS	1,4-Dioxane	60	1:3	96	72		
4d	SP382	1,4-Dioxane	60	1:3	48	64	+54.3 (2.4, MeOH)	0.29 (*C*)
4e	SP382	1,4-Dioxane	60	1:3	48	83	+42.3 (3.7, MeOH)	0.38 (*D*)
5a	SP382	Pyridine	30	1:1	48	57	+39.2 (1.0, MeOH)	0.20 (*C*)
	SP382	1,4-Dioxane	60	1:3	72	43		
	SP435	Pyridine	30	1:1	48	63		
	PS	Pyridine	30	1:2	48	38 [g]		
	PS	1,4-Dioxane	60	1:3	96	47		
5b	SP382	Pyridine	30	1:1	48	68	+14.9 (1.4, MeOH)	0.45 (*A*)
	SP382	1,4-Dioxane	60	1:3	72	58		
	SP435	Pyridine	30	1:1	48	72		
	PS	Pyridine	30	1:2	48	10 [h]		
	PS	1,4-Dioxane	60	1:3	96	51		
5c	SP382	Pyridine	30	1:1	48	81	+45.8 (0.9, MeOH)	0.42 (*E*)
	SP382	1,4-Dioxane	60	1:3	72	70		
	SP435	Pyridine	30	1:1	48	70		
	PS	1,4-Dioxane	60	1:3	96	62		
5d	SP382	1,4-Dioxane	60	1:3	72	47	+50.2 (5.0, MeOH)	0.33 (*C*)
5e	SP382	1,4-Dioxane	60	1:3	72	73	+25.0 (1.0, MeOH)	0.39 (*F*)

[a] Novo SP382 is a mixture of lipases A and B from *C. antarctica*; Novo SP435 is a cloning version of the lipase B immobilized in a polyacrylic resin; Novo SP435A is the same lipase B immobilized in Acurell; PSL is *P. cepacia* lipase. [b] Sugar-oxime derivatives **3** molar ratio. [c] Calculated with respect to the corresponding **1** or **2**. [d] Solvent *A*: 97:3 EtOAc–MeOH; solvent *B*: 97:3 CH_2Cl_2–MeOH; solvent *C*: 98:2 EtOAc–MeOH; solvent *D*: 100:1 EtOAc–MeOH; solvent *E*: 90:10 CH_2Cl_2–MeOH; solvent *F*: EtOAc. [e] Yields calculated by GC for the mixture of **4a** and **5a** (34%), once the remaining **1** was separated by flash chromatography. [f] Calculated by GC, **9b** (38%). [g] **7a** (20%) is formed as a by-product. [h] **7b** (56%) is formed as the main product.

The solvent played an essential role in the acylation of 2-deoxy-D-hexoses **1** and **2** when lipase from *Pseudomonas cepacia* was used as catalyst. In 1,4-dioxane, a

higher temperature and longer reaction times were required in order to obtain the 6-*O*-acyl derivatives **4a–c** and **5a–c**. The reaction of **1** with acetone oxime acetate (**3a**) and acetone oxime butanoate (**3b**) gave a mixture of 6-*O*- and 3-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranose (**4a** and **9a**) and, 6-*O*- and 3-*O*-butanoyl-2-deoxy-D-*arabino*-hexopyranose (**4b** and **9b**), respectively, in ~1:1 ratio, and an overall yield of 66–72% when pyridine at room temperature was the solvent. The structures of these compounds were established from the ^{13}C NMR spectra of the mixtures by comparison with data for pure samples (see later for the synthesis of **9a** and **b**). Gas chromatography of the trimethylsilyl ethers of the mixtures confirmed their compositions (see Experimental; results are summarized in Table I).

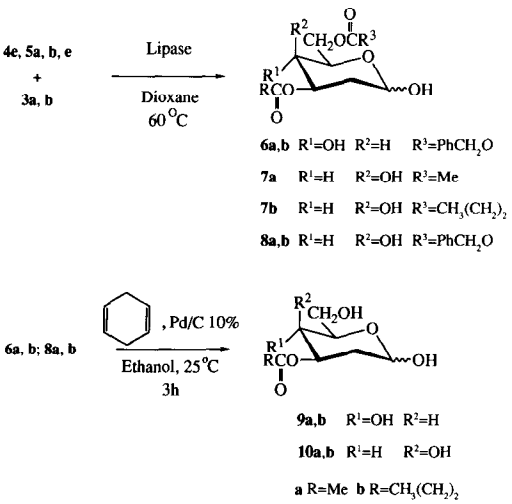
In the acylation of **2** with acetone oxime esters **3a** and **b** and *P. cepacia* lipase in pyridine, the diacylated products 3,6-di-*O*-acetyl-2-deoxy-D-*lyxo*-hexopyranose (**7a**) and 3,6-di-*O*-butanoyl-2-deoxy-D-*lyxo*-hexopyranose (**7b**) were isolated, respectively, along with the corresponding expected 6-*O*-acyl derivatives **5a** and **b** (Table I). No reactions were observed in the absence of enzyme.

Regioselective alkoxycarbonylation of 2-deoxy-D-hexoses.—As already reported[17] acetone *O*-[(alkoxy)carbonyl]oximes, 1,4-dioxane, 60°C, and *C. antarctica* lipase were suitable conditions for the formation of 6-*O*-(alkoxy)carbonyl derivatives of hexoses and pentoses.

We thought that the better solubility of 2-deoxyhexoses as compared to hexoses would permit the reaction to proceed at room temperature, but no results were obtained in preliminary screening tests at 30°C. This led us to increase the temperature to 60°C, according to the results already mentioned[17]. Under these conditions 6-*O*-(alkoxy)carbonyl derivatives **4d** and **e** and **5d** and **e** of **1** and **2** were respectively obtained in moderate yields (Table I). As with the acylation of 2-deoxy-D-hexoses **1** and **2** catalyzed by *C. antarctica* lipase, a large excess of acylating agent (up to fivefold) could be used without appreciable modification of the selectivity. The position of (alkoxy)carbonylation was confirmed by ^{13}C NMR spectroscopy with reference to the starting materials[16]. The expected downfield and upfield shifts[15] of ~6 and 2.5 ppm for C-6 and C-5, respectively, were observed (Table IV).

Regioselective acylation of secondary hydroxyl groups of 2-deoxy-D-hexoses and 1,6-anhydro-β-D-glucopyranose.—The selective acylation of secondary hydroxyl groups of carbohydrates is difficult. Only a few examples of chemo-enzymatic strategies for the selective acylation of secondary hydroxyl groups without affecting the primary one, have been reported[13,18]. In our efforts directed towards selectively acylated carbohydrates at the secondary hydroxyl groups, we have studied the acylation of the 6-*O*-(benzyloxy)carbonyl derivatives **4e** and **5e**. As the (benzyloxy)carbonyl groups[19] are readily deprotected under neutral conditions, 6-*O*-(benzyloxy)carbonyl-2-deoxy-hexoses were suitable substrates for obtaining derivatives bearing an acyl group at a secondary hydroxyl position.

Both 6-*O*-[(benzyloxy)carbonyl]-2-deoxy-D-*arabino*-hexopyranose (**4e**), and 6-*O*-



Scheme 2. Synthesis of 3-*O*-acyl derivatives of 2-deoxy-D-hexopyranoses.

[(benzyloxy)carbonyl]-2-deoxy-D-*lyxo*-hexopyranose (**5e**) were selectively acylated at HO-3 (**6a** and **b**, and **8a** and **b**, respectively) by using *P. cepacia* lipase in dioxane at 60°C (Scheme 2 and Table II).

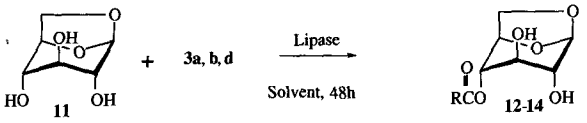
In order to confirm the structure of the diacylated compounds **7a** and **b** obtained (see foregoing) in the reaction of **2** with acetone oxime acetate (**3a**) and acetone oxime butanoate (**3b**), 6-*O*-acetyl-2-deoxy-D-*lyxo*-hexopyranose (**5a**), and 6-*O*-butanoyl-2-deoxy-D-*lyxo*-hexopyranose (**5b**) were treated under the conditions already given with *P. cepacia* lipase and excess (ratio oxime ester : sugar of 2 : 1) of acylating reagent **3a** and **3b**, respectively, yielding the corresponding diacylated products **7a** and **b** (Table V). The acylation positions were clearly established on the basis of the ^{13}C NMR spectra. The expected upfield shifts[15] of ~ 2.8 ppm for C-2 and C-4 and downfield of ~ 3.8 ppm for C-3 in the ^{13}C NMR spectra with

TABLE II

Acylation of compounds **4e**, **5a**, **b**, and **e** with acetone oxime esters **3a** and **b**

Entry	Lipase	Yield (%) [a]	$[\alpha]_D^{25}$ (*c*, solvent)	R_f (solvent) [b]
6a	PS	67	+52.3 (0.7, MeOH)	0.54 (*G*)
	SP382	34		
	SP435A	25		
6b	PS	54	+45.2 (0.5, EtOH)	0.83 (*H*)
7a	PS	63	+97.4 (0.6, MeOH)	0.47 (*F*)
7b	PS	67		0.55 (*E*)
8a	PS	56	+73.8 (0.6, MeOH)	0.75 (*G*)
8b	PS	48	+60.4 (0.5, MeOH)	0.81 (*I*)

[a] Calculated with respect to the corresponding **4** or **5**. [b] Solvent *G*: 95:5 EtOAc–Et_2O; solvent *H*: 98:2 EtOAc–Et_2O; solvent *I*: 8:2 EtOAc–Et_2O.



Scheme 3. Synthesis of 4-*O*-acyl derivatives of 1,6-anhydro-β-D-glucopyranose.

respect to the δ values for these carbon atoms in the starting materials (Table IV), indicated that the acyloxy group was at C-3.

These results agree with those obtained by Klibanov et al.[13], who showed that the acylation of the secondary hydroxyl groups of carbohydrates occurs preferably at the C-3 position when 6-*O*-butanoyl derivatives of D-glucose, D-mannose, and D-galactose were treated with 2,2,2-trihaloethyl esters. Nevertheless, we did not succeed in acylating 6-*O*-acyl derivatives of the free hexoses when oxime esters were used as acylating agents and the lipase from *P. cepacia* as catalyst[20]. *C. antarctica* lipase was less effective for the acylation of secondary hydroxyl groups and lower yields were obtained.

Deprotection of **6a** and **b** and **8a** and **b** by means of catalytic hydrogenation using 1,4-cyclohexadiene as H_2 donor and Pd on charcoal as catalyst[21] gave the expected 3-*O*-acyl derivatives **9a** and **b** and **10a** and **b**, respectively (Scheme 2). The ^{13}C NMR spectra of compounds **9** and **10** were assigned by comparison with the starting materials (Table V). Upfield shifts of ~6 ppm for C-6 and downfield shifts of ~2.4 ppm for C-5 were found as expected from the cleavage of the benzyloxycarbonyl group at C-6[15].

Enzyme-catalyzed reactions on conformationally rigid compounds are of interest in relation to the structure of the enzyme active site[9]. With this objective, regioselective deacylations on per-*O*-acylated rigid carbohydrates have been reported[14]. Selective cleavage of the 2-*O*-acyl group of per-*O*-acyl-1,6-anhydro-β-D-galactopyranose has been achieved with high selectivity using pancreatic lipase. Acylation on the conformationally rigid carbohydrate 1,6-anhydro-β-D-glucopyranose (**11**) has now been achieved. Our best results were obtained with the lipase from *C. antarctica* in 1,4-dioxane at room temperature (Scheme 3 and Table III). In this way, products **12** and **13** resulting from regioselective monoacylation at

TABLE III

Reaction of 1,6-anhydro-β-D-glucopyranose (**11**) with acetone oxime esters **3a** and **b** and acetone *O*-[(methoxy)carbonyl]oxime (**3d**)

Entry	Lipase	Solvent	T (°C)	Yield (%) [a]	$[\alpha]_D^{25}$ (*c*, solvent)	R_f (solvent)
12	SP382	Pyridine	30	70	−74.4 (1.1, MeOH)	0.48 (*C*)
13	SP382	Pyridine	30	72	−71.9 (1.0, MeOH)	0.58 (*D*)
14	SP382	Dioxane	15	42	−59.2 (1.5, MeOH)	0.49 (*F*)

[a] Calculated with respect to **11**.

TABLE IV

^{13}C NMR chemical shifts (δ_c in ppm) of products **4a–e** and **5a–e**

Compound	α-Pyranose						β-Pyranose					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
4a [a]	92.96	39.60	69.68	73.64	71.29	65.40	95.32	41.85	72.49	73.05	75.45	65.40
4b [b]	93.02	39.71	69.75	73.73	71.46	65.17						
4b [c]	92.00	37.86	68.43	71.77	70.38	63.95	94.18	40.05	70.90	71.39	74.09	64.00
4c [d]	92.71	39.40	69.42	73.42	71.13	64.87	95.10	41.63	72.28	72.86	75.34	64.87
4d [e]	93.04	39.66	69.73	73.51	71.44	68.72	95.35	42.05	72.80	73.19	75.40	68.72
4e [f]	92.86	39.40	69.59	73.29	71.25	68.62	95.20	41.65	72.36	72.73	75.30	68.62
5a [g]	93.26	34.33	66.26	69.72 [l]	69.64 [l]	65.85	95.84	37.19	69.84	68.58	74.28	65.58
5b [h]	92.13	32.49	65.06	68.30	68.30	64.95	94.50	35.34	68.90	67.23	73.20	64.74
5c [i]	93.31	34.37	66.32	69.78 [l]	69.68 [l]	65.62	95.92	37.27	69.92	68.63	74.39	65.39
5d [j]	93.18	34.12	66.08	69.51 [l]	69.45 [l]	69.04	95.74	36.99	69.64	68.34	74.00	68.72
5e [k]	93.28	34.26	66.23	69.67 [l]	69.63 [l]	69.36	95.82	37.12	69.79	68.50	74.22	69.00

[a] CD_3OD as solvent, anomeric ratio α:β, 81:19. [b] CD_3OD as solvent, no β anomer is present. [c] D_2O as solvent, anomeric ratio α:β, 76:24. [d] CD_3OD as solvent, anomeric ratio α:β, 90:10. [e] CD_3OD as solvent, anomeric ratio α:β, 90:10. [f] CD_3OD as solvent, anomeric ratio α:β, 66:34. [g] CD_3OD as solvent, anomeric ratio α:β, 90:10. [h] D_2O as solvent, anomeric ratio α:β, 57:43. [i] CD_3OD as solvent, anomeric ratio α:β, 57:43. [j] CD_3OD as solvent, anomeric ratio α:β, 60:40. [k] CD_3OD as solvent, anomeric ratio α:β, 90:10. [l] Tentative assignments.

TABLE V

^{13}C NMR chemical shifts (δ_c in ppm) of products **6a, b**; **7a, b**; **8a, b**, **9a, b**; and **10a, b**

Compound	α-Pyranose						β-Pyranose					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
6a [a]	92.75	36.98	73.24	70.41	71.35	68.52	95.90	39.50	75.38 [i]	70.00	75.94 [i]	68.52
6b [b]	91.54	34.93	71.53	69.88	70.09	66.92	93.69	38.00	72.98	69.11	74.00	66.02
7a [c]	93.26	31.47	70.53	67.12	69.36	65.85						
7b [d]	92.88	31.26	69.99	67.19	66.92	65.85	95.30	34.30	72.53	65.80	74.00	64.68
8a [c]	93.15	31.38	70.44 [i]	67.04	69.33 [i]	68.94						
8b [c]	93.21	31.52	70.23 [i]	67.18	69.45 [i]	68.95						
9a [e]	92.74	37.06	73.58 [i]	70.42	73.67 [i]	62.78	94.90	39.16	75.42	69.99	78.16	62.95
9b [f]	92.76	37.33	73.22 [i]	70.52	73.75 [i]	62.87	94.93	39.28	75.19	70.06	78.24	63.02
10a [g]	93.17	31.58	70.94	67.29	71.85	63.10	95.64	34.29	73.42	66.12	76.92	62.87
10b [h]	93.11	31.59	70.59	67.30	71.48	63.02 [i]	95.59	34.29	73.08	66.12	77.38	62.80 [i]

[a] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 90:10. [b] $CDCl_3$ as solvent, anomeric ratio $\alpha:\beta$, 85:15. [c] CD_3OD as solvent, no β anomer is present. [d] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 86:14. [e] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 67:33. [f] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 59:41. [g] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 60:40. [h] CD_3OD as solvent, anomeric ratio $\alpha:\beta$, 55:45. [i] Tentative assignments.

O-4 were obtained. At higher temperature, the selectivity decreased and complex mixtures of all possible monoacylated derivatives of **11** were obtained. Lipophilic esters could not be introduced since oxime esters bearing long chains in the acyl moiety did not react. Experiments carried out at 30 or 60°C in pyridine or 1,4-dioxane with *P. cepacia* lipase as catalyst also led to mixtures of monoacylated products.

When the structure of the substrate was changed to acetone *O*-(methoxycarbonyl)oxime (**3d**), complex mixtures of products were obtained at 60 or 30°C. Nevertheless, by lowering the temperature, the selectivity increased and 4-*O*-methoxycarbonyl-1,6-anhydro-β-D-glucopyranose (**14**) was isolated in moderate yield (Scheme 3 and Table III). The position of acylation or alkoxycarbonylation was determined according to the procedure of Yoshimoto et al.[15]. In comparison with the ^{13}C NMR spectra of the starting material[22], C-5 was shifted upfield ~ 2.4 ppm for the acyl groups and 2.6 ppm for the alkoxycarbonyl group, while corresponding signals for C-6 were downfield by 2.9 and 6.0 ppm, respectively.

EXPERIMENTAL

Materials and methods.—P. cepacia lipase, Amano PS, was purchased from Amano Pharmaceutical Co. The enzyme was kept under diminished pressure (10^{-6} mmHg) for two days prior to use. *C. antarctica* lipase, was kindly donated by Novo Nordisk A/S.

The oxime esters **3a–c** were obtained by direct reaction of the corresponding acyl chlorides with the acetone oxime, or by enzymatic methods according to procedures previously described[23]. Acetone *O*-[(alkoxy)carbonyl]oximes **3d** and **e** were obtained in almost quantitative yields by direct reaction of the corresponding alkyl chloroformates with acetone oxime in pyridine[24]. 1,4-Dioxane and pyridine were dried by distillation over $LiAlH_4$ and NaOH, respectively, and stored under N_2. TLC was performed on precoated Silica Gel 60 sheets Merck F_{254}. For flash chromatography, Merck Silica Gel 60 (230–400 mesh) was used. Solvents *A–I*, given in Tables I and II were used as eluents.

^{13}C NMR spectra were obtained using a Bruker AC 300 spectrometer at 75.5 MHz in D_2O, CD_3OD or $CDCl_3$ with Me_4Si as internal reference. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin–Elmer 1720-X FT spectrometer for KBr pellets. Elemental microanalyses were performed on Perkin–Elmer Model 240 and Carlo Erba Model 1108 instruments.

Gas chromatography.—GC analyses were carried out at 210°C isocratic temperature in a Hewlett–Packard 5890 Series II apparatus fitted with an FID detector, a split injector, and an HP 3396 Series II integrator, using N_2 as gas carrier (0.81 mL min^{-1} flow rate, split ratio 200:1). The column used was an HP 1 (25 m × 0.2 mm × 0.63 μm film thickness). Trimethylsilylated samples for GC were prepared

according to the literature procedure[25]. In the reaction of **1** with **3a** and **3b**, fractions with R_f 0.23 (mixture A) and R_f 0.41 (mixture B), were respectively separated of the remaining starting material by flash chromatography with solvent *A* as eluent. ^{13}C NMR spectroscopy of these mixtures revealed that mixture A was composed of products **4a** and **9a** and mixture B of products **4b** and **9b** in the same proportion. In order to confirm this point, GC analyses were made. The results were as follows: absolute retention times (min); relative intensity (%): **4a**, 5.86 (62%, α anomer), 6.44 (38%, β anomer). **4b**, 8.50 (65%, α anomer), 9.53 (35%, β anomer). **9a**, 5.23 (63%, α anomer), 6.08 (37%, β anomer). **9b**, 7.48 (61%, α anomer), 8.97 (38%, β anomer). Mixture A, 5.24 (29%), 5.86 (32%), 6.08 (19%), 6.44 (20%). Mixture B, 7.49 (30%), 8.51 (34%), 8.98 (18%), 9.54 (17%).

*General procedure for the synthesis of 2-deoxy-*D*-hexose monoesters (***4a–c*** *and* ***5a–c****) and of monocarbonate esters (***4d*** *and* **e** *and* ***5d*** *and* **e**).*—To the corresponding 2-deoxy-D-hexose **1** or **2** (0.41 g, 2.5 mmol) in the indicated dry solvent (25 mL), acetone oxime esters **3a–c** or acetone *O*-[(alkyloxy)carbonyl]oximes **3d** and **e**, and the corresponding lipase (0.01 g/mL for *C. antarctica*; 0.05 g/mL for *P. cepacia*) were added under N_2. The reaction was incubated in an orbital shaker at 150 rpm. Sugar-oxime derivatives **3** molar ratio, solvents, temperatures, and enzymes are detailed in Table I. Monitoring of the reaction by TLC showed that after 2–4 days, it was complete and it was then stopped by filtering off the enzyme. The crude enzyme was washed twice with MeOH (2 × 15 mL). The combined filtrate and washings were evaporated under reduced pressure, and the resulting syrup was purified by flash chromatography to yield the corresponding product (Table I):

*6-O-Acetyl-2-deoxy-*D-arabino*-hexopyranose* (**4a**).—Syrup; ν_{max} = 3403 (OH), 1726 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 173.30 (CO_α), 173.23 (CO_β), 21.07 (Me). Anal. Calcd for $C_8H_{14}O_6$: C, 46.58; H, 6.85. Found: C, 46.60; H, 6.79.

*6-O-Butanoyl-2-deoxy-*D-arabino*-hexopyranose* (**4b**).—Syrup; ν_{max} = 3395 (OH), 1711 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 177.40 (CO), 37.12 (C-2′), 19.65 (C-3′), 13.44 (Me); D_2O, δ 177.39 (CO), 36.28 (C-2′), 18.59 (C-3′), 13.43 (Me). Anal. Calcd for $C_{10}H_{18}O_6$: C, 51.26; H, 7.75. Found: C, 51.18; H, 7.69.

*6-O-Decanoyl-2-deoxy-*D-arabino*-hexopyranose* (**4c**).—Mp 80–82°C; ν_{max} = 3400 (OH), 1733 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 175.47 (CO), 34.94 (C-2′), 32.98 (C-8′), 30.53, 30.37, 30.15 (C-4′ to C-7′), 25.94 (C3′), 23.67 (C-9′), 14.46 (Me). Anal. Calcd for $C_{16}H_{30}O_6$: C, 60.34; H, 9.50. Found: C, 60.40; H, 9.41.

*6-O-[(Methoxy)carbonyl]-2-deoxy-*D-arabino*-hexopyranose* (**4d**).—Syrup; ν_{max} = 3416 (OH), 1740 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table IV and, data for the side chain, δ 157.67 (CO), 55.60 (Me). Anal. Calcd for $C_8H_{14}O_7$: C, 43.23; H, 6.35. Found: C, 43.18; H, 6.27.

*6-O-[(Benzyloxy)carbonyl]-2-deoxy-*D-arabino*-hexopyranose* (**4e**).—Syrup; ν_{max} = 3406 (OH), 1743 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table IV and data for the side

chain, δ 156.78 (CO_α), 156.70 (CO_β), 136.96 (C-1 Ph), 129.71, 129.61, 129.40 (C-2 to C-6 Ph), 70.72 (CH_2Ph). Anal. Calcd for $C_{14}H_{18}O_7$: C, 56.36; H, 6.09. Found: C, 56.56; H, 6.19.

6-O-Acetyl-2-deoxy-D-lyxo-*hexopyranose* (**5a**).—Syrup; ν_{max} = 3393 (OH), 1735 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 173.05 (CO), 21.05 (Me). Anal. Calcd for $C_8H_{14}O_6$: C, 46.58; H, 6.85. Found: C, 46.43; H, 6.87.

6-O-Butanoyl-2-deoxy-D-lyxo-*hexopyranose* (**5b**).—Syrup; ν_{max} = 3405 (OH), 1734 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 177.29 (CO), 36.27 (C-2′), 18.56 (C-3′), 13.41 (Me). Anal. Calcd for $C_{10}H_{18}O_6$: C, 51.26; H, 7.75. Found: C, 51.09; H, 7.68.

6-O-Decanoyl-2-deoxy-D-lyxo-*hexopyranose* (**5c**).—Mp 64–66°C; ν_{max} = 3446, 3357 (OH); 1732 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table IV and data for the side chain, δ 175.60 (CO), 35.20 (C-2′), 33.32 (C-8′), 30.86, 30.71, 30.50 (C-4′ to C-7′), 26.25 (C-3′), 24.01 (C-9′), 14.78 (Me). Anal. Calcd for $C_{16}H_{30}O_6$: C, 60.34; H, 9.50. Found: C, 60.23; H, 9.45.

6-O-[(Methoxy)carbonyl]-2-deoxy-D-lyxo-*hexopyranose* (**5d**).—Syrup; ν_{max} = 3393 (OH), 1744 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table IV and, data for the side chain, δ 157.43 (CO), 55.67 (Me). Anal. Calcd for $C_8H_{14}O_7$: C, 43.23; H, 6.35. Found: C, 43.14; H, 6.26.

6-O-[(Benzyloxy)carbonyl]-2-deoxy-D-lyxo-*hexopyranose* (**5e**).—Syrup; ν_{max} = 3405 (OH), 1745 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table IV and, data for the side chain, δ 156.83 (CO_α), 156.78 (CO_β), 137.17 (C-1 Ph), 129.84, 129.76, 129.54 (C-2 to C-6 Ph), 70.87 (CH_2Ph). Anal. Calcd for $C_{14}H_{18}O_7$: C, 56.36; H, 6.09. Found: C, 56.06; H, 6.00.

General procedure for the synthesis of 3,6-di-O-substituted 2-deoxy-D-*hexoses* (**6a** *and* **b**; **7a** *and* **b**; **8a** *and* **b**).—To compounds **4e**, **5a**, **b**, and **e** (1.25 mmol) in dry 1,4-dioxane (20 mL), acetone esters **3a** and **b** (2.5 mmol) and *P. cepacia* lipase (0.5 g) were added. The mixture was incubated in an orbital shaker at 60°C, 150 rpm, and monitored by TLC. After 2 days, the enzyme was filtered off and washed twice with MeOH (2 × 15 mL). The combined filtrate and washings were evaporated to dryness under diminished pressure, and the resulting syrup purified by flash chromatography to yield the corresponding products (Table II).

3-O-Acetyl-6-O-[(benzyloxy)carbonyl]-2-deoxy-D-arabino-*hexopyranose* (**6a**).—Mp 102–105°C; ν_{max} = 2948 (OH); 1743, 1730 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the 3-*O*-side chain, δ 172.70 (CO), 21.87 (Me_β), 21.40 (Me_α); data for the 6-*O*-side chain, δ 156.91 (CO), 137.28 (C-1 Ph), 129.85, 129.75, 129.53 (C-2 to C-6 Ph), 70.85 (CH_2 Ph). Anal. Calcd for $C_{16}H_{20}O_8$: C, 56.45; H, 5.93. Found: C, 56.08; H, 5.86.

6-O-[(Benzyloxy)carbonyl]-3-O-butanoyl-2-deoxy-D-arabino-*hexopyranose* (**6b**).—Mp 83–85°C; ν_{max} = 3404 (OH); 1743, 1728 cm^{-1} (C=O). ^{13}C NMR ($CDCl_3$): Table V and data for the 3-*O*-side chain, δ 174.63 (CO), 36.17 (C-2′), 18.31 (C-3′), 13.49 (Me); data for the 6-*O*-side chain, δ 155.30 (CO), 134.94 (C-1 Ph), 128.51, 128.38

(C-2 to C-6 Ph), 69.82 (CH_2Ph). Anal. Calcd for $C_{18}H_{24}O_8$: C, 58.67; H, 6.56. Found: C, 58.81; H, 6.69.

*3,6-Di-*O*-acetyl-2-deoxy-*D*-lyxo-hexopyranose* (**7a**).—Mp 109–111°C; ν_{max} = 3508, 3383 (OH), 1739, 1728 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the 3-*O*-side chain, δ 172.50 (CO), 21.36 (Me); data for the 6-*O*-side chain, δ 172.92 (CO), 21.00 (Me). Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.37; H, 6.05. Found: C, 48.29; H, 6.01.

*3,6-Di-*O*-butanoyl-2-deoxy-*D*-lyxo-hexopyranose* (**7b**).—ν_{max} = 3403 (OH); 1738, 1726 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the 3-*O*-side chain, δ 174.76 (CO), 36.98 (C-2′), 19.35 (C-3′), 13.92 (Me); data for the 6-*O*-side chain, δ 175.09 (CO), 36.67 (C-2″), 19.35 (C-3″), 13.92 (Me). Anal. Calcd for $C_{14}H_{24}O_7$: C, 55.24; H, 7.95. Found: C, 54.91; H, 7.65.

*3-*O*-Acetyl-6-*O*-[(benzyloxy)carbonyl]-2-deoxy-*D*-lyxo-hexopyranose* (**8a**).—Mp 102–104°C; ν_{max} = 3402 (OH); 1749 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the 3-*O*-side chain, δ 172.51 (CO), 21.38 (Me); data for the 6-*O*-side chain, δ 156.79 (CO), 137.22 (C-1 Ph), 129.86, 129.78, 129.54 (C-2 to C-6 Ph), 70.90 (CH_2 Ph). Anal. Calcd for $C_{16}H_{20}O_8$: C, 56.45; H, 5.93. Found: C, 56.47; H, 5.89.

*6-*O*-[(Benzyloxy)carbonyl]-3-*O*-butanoyl-2-deoxy-*D*-lyxo-hexopyranose* (**8b**).—Mp 82–84°C; ν_{max} = 3404 (OH); 1751 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the 3-*O*-side chain, δ 175.98 (CO), 37.29 (C-2′), 19.64 (C-3′), 14.21 (Me); data for the 6-*O*-side chain, δ 156.85 (CO), 137.50 (C-1 Ph), 129.88, 129.79, 129.55 (C-2 to C-6 Ph), 70.93 (CH_2 Ph). Anal. Calcd for $C_{18}H_{24}O_8$: C, 58.67; H, 6.56. Found: C, 58.72; H, 6.51.

*General procedure for the synthesis of 3-*O*-acyl-2-deoxy-*D*-hexoses (***9a*** *and* ***b*** *and* ***10a*** *and* ***b*****).*—To pure **6a** and **b** and **8a** and **b** (1 mmol) in EtOH (5 mL), 1,4-cyclohexadiene (5 mmol), and 50 mg of Pd on charcoal (10%) were added. The mixture was flushed with N_2 for 3 h. After this time, products **9a** and **b** and **10a** and **b** were obtained in quantitative yields.

*3-*O*-Acetyl-2-deoxy-*D*-arabino-hexopyranose* (**9a**).—Syrup; R_f 0.20 (solvent *C*); $[\alpha]_D^{25}$ +39.0° (*c* 1.0, MeOH); ν_{max} = 3398 (OH), 1724 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the side chain, δ 172.89 (CO_α), 172.78 (CO_β), 21.36 (Me). Anal. Calcd for $C_8H_{14}O_6$: C, 46.58; H, 6.85. Found: C, 46.51; H, 6.80.

*3-*O*-Butanoyl-2-deoxy-*D*-arabino-hexopyranose* (**9b**).—Syrup; R_f 0.41 (solvent *A*). $[\alpha]_D^{25}$ +30.3° (*c* 1.2, MeOH); ν_{max} = 3402 (OH); 1720 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table V and data for the side chain, δ 175.30 (CO_α), 175.21 (CO_β), 37.43 ($C\text{-}2'_\alpha$), 37.20 ($C\text{-}2'_\beta$), 19.69 (C-3′), 14.18 (Me). Anal. Calcd for $C_{10}H_{18}O_6$: C, 51.26; H, 7.75. Found: C, 50.81; H, 7.66.

*3-*O*-Acetyl-2-deoxy-*D*-lyxo-hexopyranose* (**10a**).—Syrup; R_f 0.15 (solvent *C*). $[\alpha]_D^{25}$ +84.2° (*c* 0.8, MeOH); ν_{max} = 3398 (OH); 1724 cm^{-1} (CO). ^{13}C NMR (CD_3OD): Table V and data for the side chain, δ 172.60 (CO_α), 172.49 (CO_β), 21.36 (Me). Anal. Calcd for $C_8H_{14}O_6$: C, 46.58; H, 6.85. Found: C, 46.27; H, 6.78.

*3-*O*-Butanoyl-2-deoxy-*D*-lyxo-hexopyranose* (**10b**).—Syrup; R_f 0.43 (solvent *A*). $[\alpha]_D^{25}$ +76.0° (*c* 0.5, MeOH); ν_{max} = 3402 (OH); 1722 cm^{-1} (C=O). ^{13}C NMR

TABLE VI

^{13}C NMR (CD_3OD) chemical shifts (δ_c in ppm) of products **12–14**

Compound	C-1	C-2	C-3	C-4	C-5	C-6
12	104.29	73.25	72.73	76.13	76.13	67.12
13	104.25	73.17	72.78	75.87 [a]	76.18 [a]	67.11
14	104.35	73.22	72.69	79.50	75.97	67.06

[a] Tentative assignments.

(CD_3OD): Table V and data for the side chain, δ 175.40 (CO_α), 175.01 (CO_β) 37.24 (C-2$'_\alpha$), 37.16 (C-2$'_\beta$), 19.60 (C-3'), 14.14 (Me). Anal. Calcd for $C_{10}H_{18}O_6$: C; 51.26; H, 7.75. Found: C, 51.36; H, 7.55.

General procedure for the synthesis of 4-O-substituted 1,6-anhydro-β-D-glucopyranose (**12–14**).—Reactions of 1,6-anhydro-β-D-glucopyranose (**11**) with compounds **3a**, **b**, and **d** using *C. antarctica* lipase were carried out according to the general procedure for the synthesis of monoesters and monocarbonates of 2-deoxy-D-hexoses using the conditions given in Table III.

4-O-Acetyl-1,6-anhydro-β-D-glucopyranose (**12**).—Mp 147–149°C; ν_{max} = 3476, 3358 (OH); 1708 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table VI and data for the acyl-side chain, δ 172.61 (CO), 21.22 (Me). Anal. Calcd for $C_8H_{12}O_6$: C, 47.04; H, 5.93. Found: C, 47.47; H, 5.91.

4-O-Butanoyl-1,6-anhydro-β-D-glucopyranose (**13**).—Syrup; ν_{max} = 3384 (OH), 1734 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table VI and data for the acyl-side chain, δ 175.11 (CO), 37.10 (C-2'), 16.94 (C-3'), 14.22 (Me). Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.70; H, 6.95. Found: C, 51.58; H, 6.79.

4-O-[(Methoxy)carbonyl]-1,6-anhydro-β-D-glucopyranose (**14**).—Syrup; ν_{max} = 3833 (OH), 1751 cm^{-1} (C=O). ^{13}C NMR (CD_3OD): Table VI and data for the acyl-side chain, δ 157.12 (CO), 55.80 (Me). Anal. Calcd for $C_8H_{12}O_7$: C, 43.62; H, 5.50. Found: C, 43.57; H, 5.41.

ACKNOWLEDGMENTS

The work was funded by CICYT (Project BIO-92-0751). R.P. gratefully acknowledges the support of the Spanish Ministery of Education and Science for a postgraduate scholarship. We also thank Novo Nordisk A/S for the generous donation of enzymes.

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