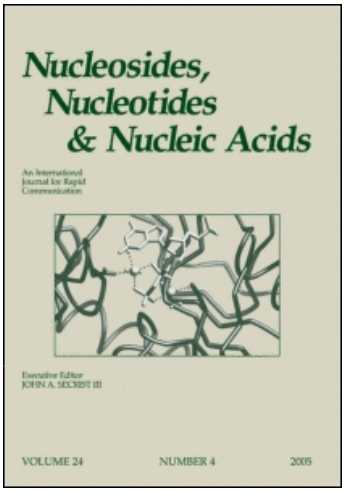

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LIPASE CATALYZED DIASTEREOSELECTIVE DEACETYLATIONS OF ANOMERIC MIXTURES OF PERACETYLATED 2'-DEOXYNUCLEOSIDES

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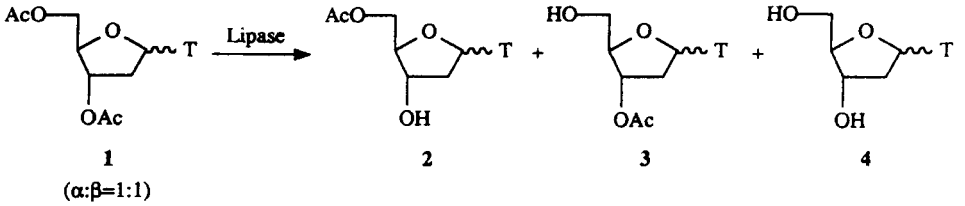
Abstract: Lipase catalyzed deacetylations of anomeric mixtures of peracetylated 2'-deoxyribofuranosyl- and 2'-deoxyribopyranosyl thymine nucleosides **1** and **5** have been investigated. Generally, the diastereoselectivity was more pronounced in pure phosphate buffer than in phosphate buffer containing 10% DMF. Wheat Germ Lipase and Porcine Liver Esterase catalyzed diastereoselective deacetylation of **1** affording the pure β-anomer thymidine (**4**β) as the only completely deprotected nucleoside product.

Selective protection and deprotection of polyfunctional molecules is a critical problem in organic synthesis. In carbohydrate and nucleoside chemistry these problems are accentuated due to the presence of multiple hydroxyl functions of very similar reactivity. The acylation and deacylation of carbohydrates using lipases has been well documented[1,2] but only few examples of lipase catalyzed biotransformations of nucleoside derivatives are known. Oxime esters have been used as irreversible acyl transfer agents in regioselective enzymatic transesterification reactions at the secondary hydroxyl group of 2'-deoxynucleosides[3] or the primary hydroxyl group of ribonucleosides.[4] Regioselective deacylations of 2'-deoxy-3',5'-di-*O*-hexanoyl pyrimidine nucleosides at the secondary hydroxyl group (lipase) or the primary hydroxyl group (protease) have been reported.[5] Other recent investigations include regioselective lipase catalyzed acylations of 5-fluorouridine[6] and deacetylations of 3',5'-di-*O*-acetylthymidine[7] and 2',3',5'-tri-*O*-acetyl-β-D-*arabino* nucleosides.[8] A common feature of the above mentioned examples is that pure β-anomers of nucleosides were used as substrates.

For the synthesis of 2'-deoxynucleoside derivatives we[9] and others[10] have used a convergent strategy, in which an activated carbohydrate is condensed with a nucleobase. This strategy allows a large number of structural variations in the carbohydrate and nucleobase moieties to be achieved, but the lack of stereoselectivity generally observed in the coupling reaction is a serious obstacle. Thus, as anomeric mixtures are obtained, laborious chromatographic separations are often required. The stereoselective introduction of nucleobases using 2'-*O*-acylated carbohydrates[11] followed by radical deoxygenation on the 2'-position[12] is one possibility to circumvent these problems. However, the extra reaction steps and the exclusion of the potentially interesting α-anomers makes this strategy less favorable. Therefore, as the first attempt to use biotransformations to solve the basic problem of separation of anomers in nucleoside chemistry, we report the first examples of diastereoselective lipase catalyzed deacetylations of anomeric mixtures of peracylated 2'-deoxyribonucleosides **1** and **5**.

Enzymes from yeast (*Candida cylindracea* Lipase, CCL), plant (Wheat Germ Lipase, WGL) and animal sources (Porcine Liver Esterase, PLE; Porcine Pancreas Lipase, PPL; Bovine Pancreas α-Chymotrypsin, α-CH) were used as catalysts in the deacetylation reactions of anomeric mixtures of 1-(3,5-di-*O*-acetyl-2-deoxy-D-ribofuranosyl)thymine (**1**) and 1-(3,4-di-*O*-acetyl-2-deoxy-D-ribopyranosyl)thymine (**5**). The diastereoselectivity of the transformations was tested in a pure aqueous medium as well as in a partly organic one.

Scheme 1 and Table 1 show the results from the lipase catalyzed deacetylations of a 1:1 mixture of α- and β-1-(3,5-di-*O*-acetyl-2-deoxy-D-ribofuranosyl)thymine **1**. WGL and PLE in pure phosphate buffer (entries 1 and 2) afforded as the only completely deprotected nucleoside product thymidine **4**β (pure β-anomer) in 29% and 31% yield, respectively. Besides, thymine (48%, WGL; 15%, PLE) and monoacetylated products **2** and **3** (11%, PLE) were isolated. These are the first encouraging examples of diastereoselective deacylations of an anomeric mixture of peracylated nucleosides. The surprising cleavage of the glycosidic linkage observed in entries 1, 2 and 4 is apparently mediated by the enzymes, as no cleavage was observed under similar conditions without enzyme. Thymine was not detected in the PPL-catalyzed reaction (entry 3), thus precluding a general protein-catalyzed (nucleophilic) mechanism for the cleavage of the glycosidic linkage. Interestingly, addition of 10% DMF to the phosphate buffer inversed the diastereoselectivity and resulted in the isolation of mixtures **4** with α:β>1 and unreacted



SCHEME 1. Lipase catalyzed deacetylations of the furanose nucleosides **1**. T = thymin-1-yl.

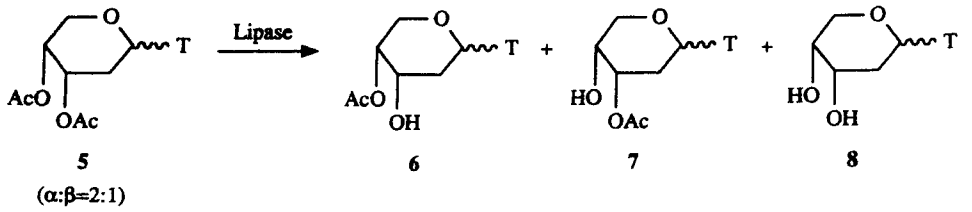
TABLE 1. Deacetylations of the furanose nucleosides. The α:β ratio of the starting material **1** was 1:1.

			Yield % (α:β)						Isolated (**1-4**)
Entry	Enzyme	Solvent	**1**		**2 + 3**	**4**		Thymine	%
1	WGL	Buffer[a]				29	(pure β)	48	77
2	PLE	Buffer			11	31	(pure β)	15	57
3	PPL	Buffer	20	(3:17)	58				78
4	α-CH	Buffer	16	(1:1)	28	15	(1:3)	25	84
5	WGL	10% DMF[b]			19	40	(3:1)		59
6	PLE	10% DMF			18	26	(2:1)		44
7	PPL	10% DMF	10	(1:1)	25	5	(4:1)		40
8	α-CH	10% DMF	22	(1:2)	39	12	(5:1)		73

[a] 0.1 M phosphate buffer, pH = 7.0.
[b] 10% DMF in 0.1 M phosphate buffer, pH = 7.0.

starting material **1** with α:β≤1. The increased transformation of **1**α in the presence of 10% DMF can be explained either by an enhanced solubility of the α-anomer relative to the β-anomer or by a conformational change in the active site of the enzymes rendering the α-anomer a comparatively better substrate than in pure phosphate buffer.

Scheme 2 and Table 2 illustrate the lipase catalyzed deacetylations of a 2:1 mixture of α- and β-1-(3,4-di-*O*-acetyl-2-deoxy-D-ribopyranosyl)thymine **5**. As above, the more diastereoselective deacetylations were obtained with WGL and PLE. WGL in pure



SCHEME 2. Lipase catalyzed deacetylations of the pyranose nucleosides **5**. T = thymin-1-yl.

TABLE 2. Deacetylations of the pyranose nucleosides. The α:β ratio of the starting material **5** was 2:1.

Entry	Enzyme	Solvent	Yield % (α:β) 5	6 + 7	8	Isolated (5-8) %
1	WGL	Buffer[a]			79 (5:1)	79
2	PLE	Buffer	33 (10:1)	21	37 (2:1)	91
3	PPL	Buffer	41 (2:1)	33	7 (2:1)	81
4	α-CH	Buffer	71 (2:1)	2		73
5	CCL	Buffer	63 (2:1)	5		68
6	WGL	10% DMF[b]		17	60 (5:1)	77
7	PLE	10% DMF	25 (3:1)	53	4 (1:1)	82

[a] 0.1 M phosphate buffer, pH = 7.0.
[b] 10% DMF in 0.1 M phosphate buffer, pH = 7.0.

phosphate buffer afforded in good yield (79%) an anomeric mixture **8** with an α:β ratio of 5:1 indicating predominantly α-deacetylation. After PLE catalyzed deacetylation in pure phosphate buffer an anomeric mixture of peracetylated starting material **5** was isolated (33% yield) with an α:β ratio of 10:1 indicating in this case a diastereoselective β-deacetylation. The pyranosyl nucleosides **5** were very poor substrates for α-CH or CCL. Addition of 10% DMF to the phosphate buffer did neither improve nor invert the diastereoselectivity (WGL and PLE) and reduced the yields of completely deacetylated **8**.

In conclusion, diastereoselective lipase catalyzed deacetylations of anomeric mixtures of peracetylated ribofuranosyl- and ribopyranosyl 2'-deoxynucleosides have been examined and achieved for the first time. The most encouraging results were obtained with the furanose nucleosides **1** as WGL and PLE catalyzed deacetylations afforded thymidine (**4**β) with complete diastereoselectivity. This suggests that biotransformations may prove useful as a novel strategy for facilitating the separation of anomeric mixtures, and we are currently investigating similar enzymatic deacylations on anomeric mixtures of nucleoside derivatives using different lipases and acyl protecting groups.

EXPERIMENTAL

All enzymes were purchased from SIGMA. Analytical TLC was carried out on silica gel 60 F_{254} plates (Merck) and short column chromatography was performed with silica gel 60 (0.040-0.063 mm, Merck).

1-(3,5-Di-*O*-acetyl-2-deoxy-D-ribofuranosyl)thymine (1)

A 1:1 mixture of α- and β-thymidine (prepared in another context as earlier reported[13]) (8.0 g, 33.0 mmol), acetic anhydride (9.3 ml, 99.0 mmol) and dry pyridine (75 ml) was stirred at room temperature for 2 h. After evaporation of the solvents under reduced pressure the residue was dissolved in chloroform (200 ml) and washed with water (3 × 50 ml). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. Purification of the crude product by short column chromatography (5% MeOH in CH_2Cl_2, v/v) afforded **1** as a white solid in 88% yield (9.5 g, 29.1 mmol, α:β = 1:1). ^1H NMR data were as reported.[14]

1-(3,4-di-*O*-acetyl-2-deoxy-D-ribopyranosyl)thymine (5)

A solution of 2-deoxy-1,3,4-tri-*O*-acetyl-D-ribopyranose[15] (13.7 g, 52.6 mmol) in dry acetonitrile (480 ml) was added in one portion to 5-methyl-2,4-bis(trimethylsiloxy)pyrimidine[16] (21.0 g, 77.6 mmol) under dry nitrogen. The mixture was cooled to −30°C and TMS-triflate (11.8 ml, 60.2 mmol) was added dropwise during 30 min. After stirring for 16 h at room temperature analytical TLC showed no more tri-*O*-acetylribopyranose, and CH_2Cl_2 (300 ml) was added and the reaction quenched with an ice-cold saturated aqueous

solution of $NaHCO_3$ (100 ml). The organic phase was successively washed with a saturated solution of $NaHCO_3$ (2 x 50 ml) and H_2O (3 x 50 ml) and dried (Na_2SO_4). After evaporation of the solvents under reduced pressure and short column chromatographic purification (5% MeOH in CH_2Cl_2, v/v), **5** was obtained as a white solid in 71% yield (12.2 g, 37.4 mmol), $\alpha;\beta$ = 2:1). ^1H NMR data were as reported.[17]

General procedure for the enzymatic deacetylations

A mixture of crude enzyme (200 mg) and **1** (or **5**) (0.36 g, 1.1 mmol) and 0.1 M phosphate buffer, pH = 7.0 (25 ml) (or 0.1 M phosphate buffer, pH = 7.0 (22.5 ml) and DMF (2.5 ml)) was stirred for 72 h at 37°C. The reaction was quenched by filtering off the enzyme over a celite pad, and the enzyme was washed with methanol. After evaporation of the solvents under reduced pressure the products were separated by short column chromatography[18] (0-10% MeOH in CH_2Cl_2, v/v) and characterized using ^1H and ^{13}C NMR spectroscopy.[19]

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18. Diacetylated (**1** or **5**), monoacetylated (**2** and **3** or **6** and **7**) or fully deprotected products (**4** or **8**) were eluted together (no attempt was made to separate anomers).
19. All compounds and mixtures were characterized by comparison of ^{1}H NMR and/or ^{13}C NMR data with published or recorded data for compounds **1**α,[14] **1**β,[14] **2**β,[20] **3**β,[3] **4**α,[21] **4**β,[22] **5**α,[17] **5**β,[17] **8**α[17] and **8**β.[17]
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