Regioselective Enzymatic Acetylation of Methyl 4,6-*O*-Benzylidene-α- and β-D-Glucopyranoside

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Lipase-catalysed acetylation of **1** furnishes the ester **3** in 94% yield while, under the same reaction conditions, the isomer **2** affords the ester **5** in 86% yield.

Carbohydrates are useful chiral synthons in natural product synthesis.[1] They contain a plethora of hydroxy groups and strategies for selective protection are often needed. Lipases have been used successfully in this respect, either to catalyse transesterification on sugars in organic media, or to promote hydrolysis of peracetylated sugars, in order to generate partially protected synthons.

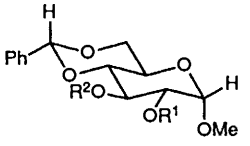
The pertinent work in this area has been reviewed recently.[2] From this compilation of data, Wong *et al.* have underlined that in both the furanose and the pyranose series, transesterifications effect the acetylation of primary hydroxy groups with high regioselectivity. In the complementary hydrolysis processes the acetylated anomeric position is the most reactive group (when present), otherwise the acetate unit on the primary hydroxy group is the preferred site of cleavage. In the last two cases complete regiospecificity can be expected.

Recently,[3,4] attention has been focussed on how lipases act, on the pyranose series, when both the primary and the anomeric hydroxy groups are masked or absent. This necessitated a study of the relative reactivities of the 2-, 3- and 4-hydroxy groups. Good to excellent 2- or 4-regioselective reactions were reported for 6-deoxy-L- and -D-hexoside and 6-*O*-butyryl α-D- and -L-galacto- and -manno-pyranoside.

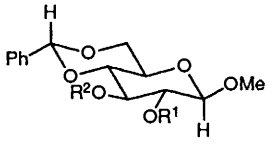
In our effort to provide very efficient and selective protection reactions in sugars we have investigated the regioselectivity of lipase-catalysed reactions on methyl 4,6-*O*-benzylidene α- and -β-D-glucopyranoside (compounds **1** and **2**).

Our method has the advantage of masking two hydroxylated positions (namely 4 and 6) which themselves exhibit very different reactivities such that the methodology can lead to the total control of all the hydroxylated positions of the hexosides.

At the start of our investigations we used the classical triad of lipases, *i.e. Pseudomonas fluorescens* lipase (PFL, Biocatalyst), porcine pancreatic lipase (PPL, Sigma) and *Candida cylindracea* lipase (CCL, Sigma). The reactions were conducted at 35 °C with enzyme (1 g) and substrate (1 g) in vinyl acetate (200 cm^3) as both the solvent and acylating agent, and with gentle stirring. The progress of the reaction was followed by TLC (ethyl acetate–toluene, 1 : 1). If the reaction was not complete within 10 days it was stopped. After removing the enzyme by filtration and evaporation of the solvent the product(s) of the reaction was (were) separated and purified by chromatography over silica. The results are summarized in Table 1.



1 $R^1 = R^2 = H$
3 $R^1 = Ac, R^2 = H$
5 $R^1 = H, R^2 = Ac$



2 $R^1 = R^2 = H$
4 $R^1 = Ac, R^2 = H$
5 $R^1 = H, R^2 = Ac$

Generally, the β-anomer was a better substrate than its α counterpart (see time and yield in Table 1). Secondly, PFL is the only lipase which catalysed relatively fast reactions with the two substrates. In addition, PPL displays complete regiospecificity with substrate **2** (formation of C-3 monoester). However, by far the most important feature in these results is the observed reversal of selectivity of PFL when changing the substrate from **1** (100% of C-2 monoester obtained) to **2** (92% of C-3 monoester obtained). As the two substrates only differ in the configuration of the anomeric position, it suggests that the substitution pattern at this position controls the regioselectivity of the PFL-catalysed reaction. This provides an unexpected and simple way to acetylate sugars selectively. Coupled to the protection–deprotection at positions 4 and 6, this exquisite control should

Table 1 Enzymatic acetylation of compounds **1** and **2**

Substrate[a]	Enzyme	Time (days)	Yield (%)	% Monoesters[b,d] C-2 **3/4**[c]	C-3 **5/6**[c]	Recovered substrate (%)
1	PPL	10	13	6	7	81
1	PFL	3	94	94	—	—
1	CCL	10	10	2	8	84
2	PPL	10	82	—	82	9
2	PFL	1	93	7	86	—
2	CCL	10	56	32	24	38

[a] Substrate **1** was purchased from Fluka A.G. Substrate **2** was synthesised from methyl β-D-glucopyranoside (Sigma) and benzaldehyde. [b] The diacetylated compounds from **1** and **2** were chemically synthesised using acetic anhydride in pyridine. In all the enzymatic reactions no diester could be detected by TLC. [c] In order to check the possibility of a thermodynamic equilibration, monoesters **4** and **6** were independently submitted to the reaction conditions (50 mg of pure monoester, 50 mg of PFL and 10 ml of vinyl acetate at 35 °C). After 1 day no interconversion was evident, as monitored by TLC. [d] The optical rotation and melting points of the products are in very good agreement with those reported earlier in the literature.[8] In all cases ^1H and ^{13}C spectra were fully consistent with the purported structures. Independent confirmation of the acetylated position (2 or 3) was given by running ^1H spectra in DMSO, revealing the coupling constant for CHOH.

allow a means of differentiating each hydroxy group in such a carbohydrate, affording the synthetic organic chemist a useful extension of protecting group methodology. Related enzyme regioselectivities have been reported previously for glycosidase[5,6] and for β-*N*-acetylhexosaminidase,[7] but to the best of our knowledge, this is the first time that the regioselectivity of a lipase is shown to be dependent on the anomeric configuration of the glucosidic acceptor.

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References

1 S. Hanessian, *Total Synthesis of Natural Products, the 'Chiron' Approach*, Pergamon, New York, 1983.
2 D. G. Drueckhammer, W. S. Hennan, R. L. Pederson, C. F. Barbas III, C. M. Gautheron, T. Krach and C. H. Wong, *Synthesis*, 1991, 499.
3 P. Cifredda, D. Colombo, F. Ronchetti and L. Toma, *J. Org. Chem.*, 1990, **55**, 4187.
4 D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, 1991, **47**, 103.
5 K. G. I. Nilsson, *Carbohydr. Res.*, 1987, **167**, 95.
6 K. G. I. Nilsson, *Carbohydr. Res.*, 1988, **180**, 53.
7 D. G. H. Crout, O. W. Howarth, S. Sing, B. E. P. Swoboda, P. Critchley and W. T. Gibson, *J. Chem. Soc., Chem. Commun.*, 1991, 1550.
8 *Dictionary of Organic Compounds*, 5th edn., Chapman and Hall; A. P. Tulloch and A. Hill, *Can. J. Chem.*, 1968, **46**, 2485.

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