

**EFFECTS OF ACYL GROUPS AND ETHANOL RATIOS ON LIPASE-CATALYZED
REGIOSELECTIVE DEACYLATION IN PERACYLATED METHYL
GLYCOPYRANOSIDES**

Kwo-Feng Hsiao^a, Fang-Lin Yang^b, Shih-Hsiung Wu*^b
and Kung-Tsung Wang*^{a,b}

^a *Department of Chemistry, National Taiwan University and*

^b *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan. R.O.C.*

Summary : Regioselective ethanolysis of peracylated methyl β , α -D-glucopyranoside and methyl α -D-mannopyranoside in anhydrous organic solvent (n-hexane/EtOH = 99/1) could afford 6-OH derivatives exclusively by *Candida rugosa* lipase (CRL). No 4 \rightarrow 6 acyl migration was observed in such an anhydrous solvent system. Substrates with propanoyl groups were more reactive than with acetyl groups on CRL-catalyzed reactions.

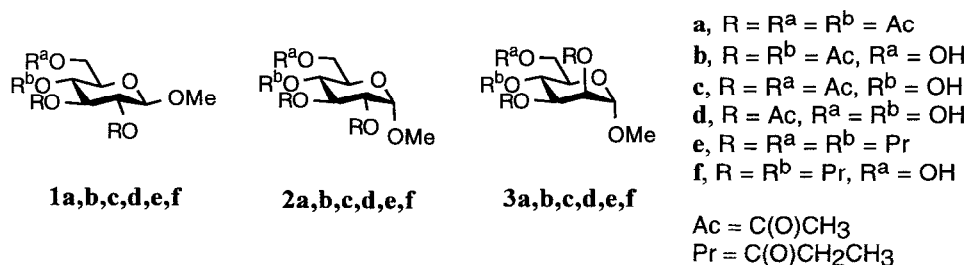
Introduction

Lipases have been proven to be effective for synthetic transformations with high selectivities by organic chemists¹ and successfully applied in carbohydrate chemistry² especially in nonpolar organic solvents. It is well-known that the catalytic efficiencies and selectivities of lipase are affected by both substrate structure and solvent hydrophobicity³. Regioselective protection of carbohydrate is a considerable challenge because the presence of multiple hydroxyl groups of similar reactivity. Partially acylated sugars are of great importance and widespread application in carbohydrate chemistry⁴. In previous work, partially acylated sugars have been prepared by lipase-catalyzed regioselective deacylations^{5,6} and acylations^{6,7}.

One disadvantage was that in the enzymatic reaction in a buffer system, partially acetylated glycopyranosides from the lipase-catalyzed reaction undergo acetyl migration⁸ so that column chromatography is necessary to separate the mixtures.

To the best of our knowledge, no studies have been carried out to elucidate the effect of substrate structure and ethanol ratios on the catalytic efficiency of lipase-catalyzed reaction. Modifying both the substrate structure and the reaction medium were employed to

optimize enzyme-catalyzed procedure. CRL (*Candida rugosa* lipase) catalysis could be controlled by the hydrophobicity of the solvent and the structure of the acyl moiety⁹. Acetyl migration could be ignored in the solvent with low polarity and without salts. We, therefore, studied the modification of this enzyme-catalyzed procedure, changing the solvent hydrophobicity by adjusting ethanol ratios in n-hexane, and changing the substrate structure by regulating the chain length of acyl groups on monosaccharides to develop a novel, efficient and simple methodology to obtain 6-OH sugar acetates via "substrate engineering" and "medium engineering".



Experimental Section

¹H NMR and ¹³C NMR spectra were recorded on a Bruker AM-400 instrument in CDCl₃ and are reported in δ units relative to CHCl₃ fixed at 7.24 ppm (¹H) and 77 ppm (¹³C). Analytical TLC was performed on precoated MERCK 60 HF₂₅₄ silica gel G plates (0.25 mm thickness); spots were visualized by spraying with 5% sulfuric acid in ethanol and heated on hot plate. Column chromatographies were performed with Merck 60 silica gel (70-230 mesh) by Still method (flash chromatography). HPLC analyses were performed on a Hitachi L-6200A intelligent pump system. The analyses were carried out on a reverse-phase Nucleosile C-18 column (10 μ m; 4.6x250mm), with the solvent mixture of CH₃CN and H₂O; the flow rate was 1 mL \cdot min⁻¹, and detection was performed at 214 nm.

CRL (*Candida rugosa* lipase) was purchased from Sigma Chemical Co. In all experiments, the lipase was used directly from the bottle without any prior pretreatment. All organic solvents were of analytical grade and, prior to use, were stored on 4-Å molecular sieves (Merck) (about 1 g of molecular sieves/5 mL of solvent) at room temperature. The molecular sieves employed in this work was activated in an oven for 48 hour at 120 °C. All other reagents not mentioned were used directly from the bottles.

Structure Determination :

All the structure determination were performed by NMR and the position of deacylation in all enzymatically prepared compounds was established by ¹H NMR and ¹³C NMR which compared the corresponding fully peracetylated sugars. At the 6-position, the chemical shift of CH₂ at the deacylation site would show an up-field shift 0.5 ~ 0.7 ppm relative to the corresponding acylation site.

1. General Procedure for CRL-Catalyzed Hydrolysis of peracetylated sugars 1a, 2a and 3a in Phosphate Buffer (Table 1.)

The substrate (1 g) was stirred in a 50 ml phosphate buffer solution (0.1 M pH 7.0). After 2 g enzyme is added, the mixture was stirred at room temperature, and the reaction is monitored by TLC. The reaction was kept at pH 7.0 with 1 N NaOH. Once the substrate was consumed, the reaction was terminated and extracted by EtOAc (3 x 100 mL). Then, all the products were purified by column chromatography with the eluent from n-hexane-diethyl ether (2:8) to diethyl ether.

2. General Procedure of CRL-Catalyzed Ethanolysis of Peracetylated Sugars 1a, 2a and 3a in Organic Solvent

The substrate (181 mg, 0.5 mmol) was suspended in 10 mL solvent mixture (n-hexane-ethanol, 99:1). After 0.5 g enzyme was added, the mixture was shaken at 240 rpm at 30°C, and the reaction was analyzed by HPLC for the conversion by withdrawing 0.1 mL reaction solution in each interval, then filtration of the enzyme and evaporation of the solvent in a stream of nitrogen. The crude compounds were analyzed quantitatively by dissolving in 0.2 mL acetonitrile for HPLC. The analysis from HPLC was carried out in the program, 1 mL•min⁻¹ 20 % CH₃CN. Once the required extent of conversion was reached, the enzyme was filtered off, washed with Et₂O and the solvent removed by evaporation and the reactions stopped after 48 h. If necessary, the hydroxyl products and the unreacted tetraesters were separated by chromatography on a silica gel column and with hexane-ethyl acetate (1:1) as a eluent.

3. General Procedure of CRL-Catalyzed Ethanolysis of Perpropanoylated Sugars 1c, 2c and 3c in Organic Solvent

The substrate (209 mg, 0.5 mmol) was dissolved in 10 mL solvent mixture (n-hexane-ethanol, 99:1), and process as above (2). The analysis by HPLC was carried out at 1 mL•min⁻¹ 42 % CH₃CN. If necessary, the hydroxyl products and the unreacted tetraesters were separated by chromatography on a silica gel column and with hexane-ethyl acetate (2:1) as a eluent.

Table 1. CRL-Catalyzed Hydrolysis of Peracetylated Methyl Glycopyranosides in a Phosphate buffer.^a

entry	substrate	reaction time (h)	products (yield, %) ^b		
1	1a	12	1b (30)	1c (11)	1d (58)
2	2a	2	2b (91)	2c (3)	2d (3)
3	3a	2.5	3b (94)	3c (3)	3d (2)

a, Reaction conditions are described as in the experimental section. b, Isolated yield.

Results and Discussion :

In general, lipases are well-known to catalyze hydrolysis of peracetylated carbohydrates in aqueous solutions with high regioselectivities². Several commercial lipases were screened and are listed in **Table 1**. To effect lipase-catalyzed regioselective hydrolysis of peracetylated methyl glycosides **1a**, **2a** and **3a**, *Candida rugosa* lipase (CRL) was a powerful catalyst for the preparation of partially acetylated monosaccharides with one hydroxyl group on primary position in phosphate buffer (0.1 M, pH 7.0). CRL is well-

known as a highly useful catalyst for regioselective reactions in organic solvents^{1,10} and the catalytic efficiency can be changed as the chain length of acyl groups changed^{9,5(a),11}. Unfortunately, some drawbacks are that 4-OH derivatives were formed from 6-OH derivatives via 1,3-acetyl migration¹² in the buffer solution and, furthermore, 4,6-OH derivatives are generated from CRL-catalyzed cleavage of 6-O-acetyl group of 4-OH derivatives⁸. It was therefore necessary to purify the mixtures by column chromatography. This moved us to adjust the reaction system to avoid the acetyl migration.

Table 2. CRL-Catalyzed Ethanolysis of **3e** in Various Ratio of Ethanol in n-Hexane^a

entry	solvent	product yield (%)			initial rate ^b
	ratio of ethanol in n-hexane (%)	5 h	9 h	24 h	10 ³ (mM•h ⁻¹)
1	0.5	41.0	41.7	43.3	18
2	1	53.4	67.2	84.0	11
3	2	48.4	59.5	72.6	10.2
4	5	22.7	36.5	66.4	4.4
5	10	10.3	19.1	41.8	1.2
6	20	2.1	5.1	12.4	0.2
7	50	0	0	0.3	0
8	100	0	0	0	0

a, Reaction condition: each substrate (0.5 mmol) was dissolved in 10 ml of anhydrous solvent mixture (listed in the column 2), followed by addition of *C. rugosa* lipase (500 mg). The further analysis was described as the experimental section. c, The initial rate is determined in catalyzed reaction for 0.5 h.

Compound **3e** was selected as a model substrate and the reaction medium was composed with n-hexane and ethanol as an acyl acceptor. Perpropanonylated methyl α -D-mannopyranoside **3e** could be dissolved in all the solvent mixtures tested. CRL catalysis was performed for the examination of the effect of ethanolysis in different ratio between ethanol and n-hexane, the results shown in **Table 2**.

As expected, CRL catalyzed regiospecific ethanolysis was on the 6-acyl group and only one product was produced without any other compounds monitored from TLC and HPLC. The catalytic efficiency of CRL-catalyzed ethanolysis is highly dependent on the hydrophobicity of the reaction medium. So, the solvent mixture (n-Hexane/EtOH =99/1) showed the most suitable reaction medium and was selected for further investigation.

The general reaction condition was established from **Table 2.**, and two different chain lengths of acyl groups were introduced methyl β or α -D-glucopyranoside and methyl α -D-mannopyranoside for the further study. The reaction progress were analyzed by HPLC¹³ and all the reactions were stopped after incubation for 48 hours. All the results and typical procedure are shown in **Table 3.**, only 6-OH derivatives were found without any other products. The structure of products were determined by ¹H and ¹³C NMR spectra compared to the established data from partially acetylated methyl glycopyranosides in the related work^{5e, 5g, 6a.}

Table 3. CRL-Catalyzed Regioselective Ethanolysis of Peracylated Methyl Glycopyranosides in Anhydrous Media^a

entry	substrate	product	yield (%) ^b		initial rate ^d
			24 h	48 h	10 ³ (mM•h ⁻¹)
1	1a	1b	38	84 ^c	9.6
2	1e	2f	71	100	35
3	2a	2b	100	-	30.4
4	2e	2f	100	-	88.8
5	3a	3b	60	94	21.6
6	3e	3f	73	100	69.1

a, The acylated compounds were chemically synthesized using acid anhydride and perchloric acid as the catalyst. Reaction conditions are as described as the experimental section. b, The yield was calculated by integration peak area from HPLC. c, Additional 0.1 mL ethanol added in the reaction for 24 h, the yield was calculated in the reaction stopped (48 h). d, The initial rate was based on the reaction after 3 h.

From **Table 3.**, the acyl group on primary position is the only site for *C. rugosa* lipase-catalyzed ethanolysis, and the catalytic efficiencies are largely influenced by the orientation of substituted groups as well as the chain length of acyl groups. Generally, the α -conformers are better substrates than the β -conformers, which was similar to being reacted in phosphate buffer (**Table 1.**). The substrates with propanoyl groups are more reactive than with acetyl groups for CRL-catalyzed regioselective ethanolysis reactions. It was worthy to note that the accumulation of 6-OH derivatives of methyl β -D-glucopyranoside were largely increased because no migration occurred.

Conclusion

In conclusion, the lipase from *Candida rugosa* (CRL) is an effective biocatalyst for the regioselective ethanolysis of peracylated methyl glycopyranosides in nonpolar organic solvents. The CRL-catalyzed ethanolysis of peracylated methyl glycopyranosides can be not only efficiently but also simply performed in such a solvent system and it is not

necessary to purify the product by column chromatography. From the view of organic synthesis, the suitable regulatory of acyl groups on sugar for enzyme-catalyzed ethanolysis in nonpolar solvent can open another way to promote and modify the enzyme application in carbohydrate chemistry.

References :

1. (a) Chen, C. S.; Sih, C. J. *Angew. Chem. Int. Eng. Ed.*, **1989**, *28*, 695.
(b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114.
2. (a) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, III, C. F.; Gautheron, C. M.; Krach, T; Wong, C. H. *Synthesis*. **1991**, 499.
(b) Wong, C. H.; Whiteside G. M. *Enzymes in Synthetic Organic Chemistry*, 1st ed.; Elsevier; New, York, 1994.
3. Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253.
4. (a) Haines, A. H., *Adv. Carbohydr. Chem. Biochem.*, **1976**, *33*, 11.
(b) Haines, A. H., *Adv. Carbohydr. Chem. Biochem.*, **1981**, *39*, 13.
5. (a) Sweers, H.M.; Wong, C.H., *J. Am. Chem. Soc.* **1986**, *108*, 6421.
(b) Chang, K.Y.; Wu, S.H.; Wang, K.T., *Carbohydr. Res.*, **1991**, *222*, 121.
(c) Chang, K.Y.; Wu, S.H.; Wang, K.T., *Carbohydr. Res.*, **1991**, *222*, 121.
(d) Chang, K.Y.; Wu, S.H.; Wang, K.T., *J. Carbohydr. Chem.*, **1991**, *10*, 251.
(e) Wu, S. H.; Ong, G. T.; Hsiao, K. Y.; Wang, K. T. *J. Chin. Chem. Soc.* **1992**, *39*, 675.
(f) Ong, G.T.; Chang, K.Y.; Wu, S.H.; Wang, K.T., *Carbohydr. Res.*, **1993**, *241*, 327.
(g) Hsiao, K.F.; Wu, S.H.; Wang, K.T., *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2125.
(h) Ong, G.T.; Chang, K.Y.; Wu, S.H.; Wang, K.T., *Carbohydr. Res.*, **1994**, *265*, 311.
(i) Chen, H. P.; Hsiao, K.F.; Wu, S.H.; Wang, K.T., *Biotechnol. Lett.* **1995**, *17*, 305.
6. (a) Hene, W. J.; Sweers, H. M.; Wang, Y.-F; Wong, C.H. *J. Org. Chem.* **1988**, *53*, 4939.
(b) Holla, W. *Angew. Chem. Int. Engl. Ed.* **1989**, *28*, 220.
7. (a) Riva, S.; Chopineau, J.; Kieboom, A.P.G ; A.M. Klibanov, *J. Am. Chem. Soc.* **1988**, *110*, 584.
(b) Therisod, M.; Klibanov, A.M. *J. Am. Chem. Soc.* **1987**, *109*, 3977.
8. Hsiao, K.F.; Lin, H. J.; Leu, D. L.; Wu, S.H.; Wang, K.T., *Bioorg. Med., Chem. Lett.* **1994**, *4*, 1629.
9. Parida, S.; Dordick, J. S. *J. Org. Chem.* **1993**, *58*, 3238.
10. (a) Laane, C.; Tramper, J.; Lilly, M. D. *Biocatalysis in Organic Media*, ed.; Elsevier: Amsterdam, 1986.
(b) Chen, C. H.; Wu, S. H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.
(c) Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* **1988**, *53*, 2374.
11. Stamatis, H.; Xenakis, A.; Provelegiou, M.; Kolisis, F. N. *Biotechnol. Bioeng.* **1993**, *42*, 103.
12. Liu, K. K. C.; Nozaki, K.; Wong, C. H. *Biocatalysis* **1990**, *3*, 169.
13. From the chromatograms, only the 6-OH derivatives and the corresponding ethyl ester were new-observed.