**SELECTIVE MONOACETYLATION OF DIOL COMPOUNDS BY
ASPERGILLUS NIGER LIPASE**

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Summary : The primary monoesters of diol compounds were formed exclusively in the reaction with vinyl acetate and *Aspergillus niger* lipase for 24~72 h. Various diol compounds which included 1,3-butanediol, 1,4-butanediol, 1,5-hexanediol, 1-phenyl-1,2-ethanediol, 1-phenyl-1,3-propanediol, 2, 3, or 4-hydroxybenzyl alcohol, methyl 2, 3-*O*-acetyl-D-glycopyranosides and phenyl 1-thio-β-D-xylopyranoside have been examined and showed nearly 100% regioselectivity.

Introduction

Selective acylation of one of two hydroxy groups in the diol compounds is an important step in organic synthesis (Kocienski, 1994). It is a challenge to develop a method for differentiating two hydroxy groups of diol compounds by direct monoacylation.

A number of chemical methods have been established for the selective acylation of primary hydroxy group of the diol compounds with specially activated acyl donors, amine bases or complex catalyst (Yamada, 1992; Ishihara *et al.* 1993). However, in these cases, a lower temperature or vigorous reaction conditions were required, and column chromatography had to be performed because of the mixtures resulted from the poor regioselectivities or diacetylated products formed in prolonged reaction time. In our approach, we developed an extremely simple, cheap, and highly regioselective method for obtaining primary monoacetates of diols.

Lipases have widespread applications in organic synthesis because of low cost, versatile nature, easy use and non-requirement of added cofactors (Chen & Sih, 1989; Klivanov 1990; Wong & Whiteside, 1994). Their regioselective capabilities have also been recognized in different hydroxy groups within the same molecule in the case of carbohydrate (Druekhammer *et al.* 1991), steroids and glycols (Cesti *et al.* 1985; Janssen *et al.* 1991; Parmar *et al.* 1993). In our laboratory, lipase-catalyzed deacetylation in polyacetylated compounds have successfully differentiated primary and secondary position (Chang *et al.* 1991; Wu *et al.* 1992; Ong *et al.* 1993; Hsiao *et al.* 1993; Ong *et al.* 1994; Chen *et al.* 1995; Hsiao *et al.* 1995; Hsiao *et al.*

1996). Based on the previous results, we extend the application of lipase-catalyzed acetyl transfer for the monoacetylation of primary hydroxyl group of diol compounds by using vinyl acetate as acetyl donor and solvent (**Figure 1**). Vinyl acetate is an inexpensive reagent and especially suitable for irreversible enzymatic acetyl transfer reaction (Wang & Wong, 1988).

Figure 1. The structures of diol compounds and monosaccharide derivatives.

a $R^1 = R^2 = H$ **b** $R^1 = OAc, R^2 = H$ **c** $R^1 = OAc, R^2 = OAc$

Table 1. Characteristic chemical-shift region of 1H NMR.

(a).	CH_2OH	$CHOH$	CH_2OAc	$CHOH$	CH_2OAc	$CHOAc$		
3a	3.58	4.01	3b	3.89	3.57	3c	3.84	5.01
4a	3.81	3.62	4b	4.05	3.80	4c	4.03	4.90
5a	3.60	3.76	5b	4.03	3.64	5c	4.01	4.86
6a	3.64, 3.74	4.80	6b	4.23, 4.14	4.93	6c	4.23, 4.32	5.99
7a	3.84	4.96	7b	4.11	4.75	7c	4.27	5.83

(b).	CH_2OH		CH_2OAc	
	8a	4.83	8b	5.10
	9a	4.61	9b	5.03
	10a	4.35	10b	4.93

Experimental

General. : ^1H NMR spectra were recorded on a Bruker AM-400 instrument in CDCl_3 and were reported in δ units relative to CHCl_3 fixed at 7.24 ppm. TLC was performed on precoated silica gel G plates (MERCK, HF_{254}).

Aspergillus niger lipase was commercially available from Amano (Japan) and used without further purification. All the structures determination were performed by NMR and the position of acylation in all enzymatically prepared compounds were established by ^1H NMR which compared the corresponding diols and diacetates (Table 1). Vinyl acetate and diisopropylether were used without further purification apart from drying by shaking with 4 Å molecular sieves.

General Procedure for Lipase-Catalyzed Acetylation of Diols (3a, 4a and 5a) with Vinyl Acetate in Diisopropylether.

The diol substrate (10 mmol, 0.9 g for 3a, 1.04 g for 4a, 1.18 g for 5a) and vinyl acetate (25 ml) were dissolved in diisopropylether (25 ml). After 2 g *Aspergillus niger* lipase was added, the mixture was shaken at room temperature, and the reaction was monitored by GLC and NMR for the conversion by withdrawing 2 mL reaction solution in each interval, 1.8 ml for NMR and 0.2 mL for GLC respectively. Once the required extent of conversion was reached, the enzyme was filtered off and the solvent was removed by evaporation in a stream of nitrogen. All the structures were determined in a crude mixture and compared with authentic compounds. GLC for the separation of the relating derivatives with a Chromosorb 5-ft WHP column with 5% OV-101 as the stationary phase. Carrier gas N_2 , 30 $\text{mL}\cdot\text{min}^{-1}$, injector temperature 60 °C, 100 °C, 3 °C $\cdot\text{min}^{-1}$, 200 °C. Retention time (min); 3a (13.86), 3b (10.81), 3c (8.74), 4a (16.37), 4b (14.56), 4c (12.25), 5a (19.73), 5b (17.56), 5c (15.56). Selected characteristic ^1H NMR : 1-*O*-acetyl-1,4-pentanediol (4b); δ_{H} (400 MHz, CDCl_3) 1.09 (d, 3H), 1.32~1.71 (m, 4H), 1.94 (s, 3H, COCH_3), 2.59 (s, 1H, OH), 3.80 (m, 1H), 4.05 (t, 2H). 1,4-*O*-diacetyl-1,4-pentanediol (4c); δ_{H} (400 MHz, CDCl_3) 1.12 (d, 3H), 1.34~1.72 (m, 4H), 1.94, 1.95 (2s, 6H, COCH_3), 4.03 (t, 2H), 4.90 (m, 1H). 1-*O*-acetyl-1,5-hexanediol (5b); δ_{H} (400 MHz, CDCl_3) 1.03 (d, 3H), 1.23~1.59 (m, 6H), 1.90 (s, 3H, COCH_3), 2.77 (s, 1H, OH), 3.64 (m, 1H), 4.03 (t, 2H). 1,5-*O*-diacetyl-1,5-hexanediol (5c); δ_{H} (400 MHz, CDCl_3) 1.07 (d, 3H), 1.32~1.70 (m, 6H), 1.91, 1.94 (2s, 6H, COCH_3), 4.01 (t, 2H), 4.86 (m, 1H).

General Procedure for Lipase-Catalyzed Acetylation of Diols (6a and 7a) with Vinyl Acetate in Diisopropylether.

The diol substrate (10 mmol, 1.38 g for 6a, 1.52 g for 7a) and vinyl acetate (25 mL) are dissolved in diisopropylether (25 mL). After 2 g *Aspergillus niger* lipase was added, the mixture was stirred at room temperature, and the reaction was monitored by NMR for the conversion by withdrawing 2 mL reaction solution in each interval for ^1H NMR. Once the required extent of conversion was reached, the enzyme was filtered off and the solvent was removed by evaporation in a reduced pressure at 30 °C.

General Procedure for Lipase-Catalyzed Acetylation of Diols (8a, 9a and 10a) with Vinyl Acetate in Diisopropylether.

The diol substrate (10 mmol, 1.24 g) and vinyl acetate (25 mL) are dissolved in diisopropylether (25 mL). After 2 g *Aspergillus niger* lipase was added, the mixture was shaken at 240 rpm at room temperature, and the reaction was monitored by ^1H NMR for the conversion by withdrawing 2 mL reaction solution in each interval for NMR analysis. Once the required extent of conversion was reached, the enzyme was filtered off and the solvent was removed by evaporation in a reduced pressure at 30 °C.

General Procedure for Lipase-Catalyzed Acetylation of Diols (11a, 12a and 13a) with Vinyl Acetate in Diisopropylether.

The diol substrate (0.5 mmol, 140 mg) and vinyl acetate (5 mL) was dissolved in diisopropylether (5 mL). After 0.5 g *Aspergillus niger* lipase was added, the mixture was shaken at 240 rpm at room temperature, and the reaction was monitored by HPLC for the conversion by withdrawing 0.2 mL reaction solution in each interval. Once the required extent of conversion was reached, the enzyme was filtered off and the solvent was removed by evaporation. The peaks assignment and area were studied with the established compounds (Hsiao *et al.* 1993). HPLC analyses were performed on a Hitachi L-6200A intelegent pump system. The analyses were carried out on a reverse-phase Lichrosorb C-18 column (10 μm ; 4.6x250mm), with the solvent mixture of 20% $\text{CH}_3\text{CN}_{(\text{aq})}$; the flow rate was 1 $\text{mL}\cdot\text{min}^{-1}$, and detection was performed at 214 nm. Retention time (min) : 11a (5.8), 11b (12.3), 12a (5.4), 12b (11.4), 13a (5.7), 13b (12.0). Selected characteristic ^1H NMR : methyl 2,3,4-*O*-triacetyl- β -D-glucopyranoside (11b); δ_{H} (400 MHz, CDCl_3) 1.97,

1.99, 2.01 (3s, 3H, COCH₃) 3.40 (s, 3H, OMe), 3.48 (ddd, 1H, H-5, $J_{5,6} = 1.7$ Hz, $J_{5,6'} = 3.9$ Hz), 3.54 (t, 1H, H-4, $J_{4,5} = 9.1$ Hz), 4.28 (dd, 1H, H-6, $J_{6,6'} = 12.2$ Hz), 4.36 (d, 1H, H-1, $J_{1,2} = 9.5$ Hz), 4.39 (dd, 1H, H-6'), 5.25 (t, 1H, H-3, $J_{3,4} = 8.6$ Hz). methyl 2,3,4-*O*-triacetyl- α -D-glucopyranoside (**12b**); δ_H (400 MHz, CDCl₃) 2.06, 2.07, 2.13 (3s, 3H, COCH₃), 3.29 (br, 1H, OH), 3.48 (s, 3H, OMe), 3.52 (bt, 1H, H-4, $J_{4,5} = 9.6$ Hz), 3.78 (ddd, 1H, H-5, $J_{5,6} = 2.3$, $J_{5,6'} = 4.7$ Hz), 4.10 (br, 1H, H-4), 4.26 (dd, 1H, H-6, $J_{6,6'} = 12.1$ Hz), 4.37 (dd, 1H, H-6'), 4.79 (dd, 1H, H-2, $J_{2,3} = 10.0$ Hz), 4.84 (d, 1H, H-1, $J_{1,2} = 3.7$ Hz), 5.25 (t, 1H, H-3, $J_{3,4} = 9.4$ Hz). methyl 2,3,4-*O*-triacetyl- α -D-mannopyranoside (**13b**); δ_H (400 MHz, CDCl₃) 2.11, 2.17, 2.18 (3s, 3H, COCH₃), 2.87 (br, 1H, OH), 3.47 (s, 3H, OMe), 3.77 (br, 2H, H-4, H-5), 4.28 (dd, 1H, H-6, $J_{5,6} = 1.4$ Hz, $J_{6,6'} = 12.1$ Hz), 4.48 (dd, 1H, $J_{5,6'} = 4.6$ Hz, H-6'), 4.64 (d, 1H, H-1, $J_{1,2} = 1.5$ Hz), 5.15 (m, 2H, H-2, H-3).

General Procedure for Lipase-Catalyzed Acylation of Phenyl 1-thio- β -D-Xylopyranoside **14 with Vinyl Acetate.**

The substrate **14** (20 mg) and *Aspergillus niger* lipase (200 mg) were mixed in 5 mL vinyl acetate. The followings are described as above mentioned. There was no reaction occurred for reaction 24 h.

Results and Discussion

Simple aliphatic alcohols, such as 1-hexanol (**1a**), 2-hexanol (**2a**) and 1,5-hexanediol (**5a**), were used initially as the model substrates for lipase screening. As shown in **Scheme 1**, compound **1a** could be converted into the corresponding acetate by *Aspergillus niger* lipase-catalyzed transesterification in vinyl acetate solution; while no corresponding acetate was formed in the case of compound **2a** in the same reaction condition for 36 h. As for 1,5-hexanediol (**5a**), only corresponding primary ester was formed after reaction for 36 h. The results strongly indicated that primary and secondary hydroxyl groups in diol compounds might be differentiated by *Aspergillus niger* lipase-catalyzed acetylation in vinyl acetate solution and corresponding primary monoacetates could be obtained. Therefore, in order to establish a general regioselectively monoacetylated method, several different diol compounds have been tested by the reaction of *Aspergillus niger* lipase-catalyzed transesterification.

Scheme 1. *Aspergillus niger* lipase-catalyzed selective acetylation of primary alcohol.

Reaction conditions : a mixture of an alcohol (5 mmol, 511 mg for **1a** and **2a**, 590 mg for **5a**) and *Aspergillus niger* lipase (1 g) in vinyl acetate (20 mL) was shaken at 240 rpm at room temperature. Aliquots was withdrawn from the reaction mixture for the analysis by GLC at different time intervals. The analysis was the same as the description in the experimental section.

As expected, it was observed that primary monoesters were formed quantitatively relative to the conversion of diol compounds by *Aspergillus niger* lipase-catalyzed transesterification. The results are listed in **Table 2**. The common feature in these reactions is

that the substrates were acetylated exclusively on primary hydroxyl position and no other side products were observed. The structure of the products are easily identified by comparing the signals of CH_2OH and CH_2OAc of substrates and products in ^1H NMR spectra. In the series of aliphatic diols (**3a**, **4a** and **5a**), they are different in the chain length between primary and secondary hydroxy groups. Free diols could be converted into primary monoacetates in 12 h reaction and no diacetate or secondary monoacetate could be detected in our study by GLC analysis. When the reaction time was prolonged until 48 h, only trace amounts of diacetate appeared in the chromatogram of GLC analysis. It implied that the moiety of secondary alcohols difficultly entered the active site of *Aspergillus niger* lipase.

The same reaction system was also examined with 1-phenyl-1,2-ethanediol (**6a**) and 1-phenyl-1,3-propanediol (**7a**). The corresponding primary monoacetates were obtained as well.

Table 2. *Aspergillus niger* lipase-catalyzed selective acetylation of diol compounds on primary position with vinyl acetate in organic solvent.

Substrate	Time (hour)	Compounds Distribution (%)		
		Diol	: Monoester ^a	: Diester
3a	24	0	98	2
4a	24	0	98	2
5a	24	0	98	2
6a	72	0	100	0
7a	72	5	95	0
8a	48	7	93	0
9a	48	11	89	0
10a	48	23	77	0
11a	48	37	63	0
12a	48	24	76	0
13a	48	29	71	0
14	24	-	-	-

^a Only primary monoester formed.

It is interesting that 2, 3, or 4-hydroxybenzyl alcohol (**8a**, **9a**, **10a**) which reacted with *Aspergillus niger* lipase in vinyl acetate was transformed into the benzyl acetate derivatives, respectively. The higher reaction rate was observed in the substrate whose primary hydroxy group was closer to the phenyl ring. It may imply that the phenyl ring in the substrate hydrobically interacts with *Aspergillus niger* lipase molecule adjacent to the active site and somehow enhance the enzymatic reaction.

In order to evaluate the application of *Aspergillus niger* lipase-catalyzed transesterification to carbohydrate molecules, three partially protected monosaccharides (**11a**, **12a** and **13a**) were also tested. Only the primary hydroxy group (6-OH) could attack the acetyl enzyme intermediates to form acetate. Although their reaction rate was much slower than aliphatic diol compounds, the catalytic efficiency and regiospecificity was enough to get the derivatives of monosaccharides at preparative scale. Phenyl 1-thio- β -D-xylopyranoside (**14**) with three secondary hydroxyl groups was also examined as a substrate. There was no acetate products formed in 24 h. It gave more proof that *Aspergillus niger* lipase hardly accepted secondary hydroxyl group as a substrate in acetyl transfer reaction.

Only the primary monoester is produced quantitatively relative to the conversion of the starting diol. In conclusion, it is believed that *Aspergillus niger* lipase-catalyzed acetyl transfer can be used as an efficient, simple and mild method to selectively catalyze the formation of primary acetate derivatives from diol compounds in excellent yields.

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