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Chemoenzymatic synthesis of *d*-biotin intermediate lactone via lipase-catalyzed desymmetrization of meso diols

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ABSTRACT

A chemoenzymatic methodology for the asymmetric synthesis of *d*-biotin intermediate lactone ((3a*S*, 6a*R*)-tetrahydro-1,3-dibenzylhexahydro-1*H*-Furo[3,4-d] imidazole-2,4-dione) **1** has been demonstrated. The key step of the synthetic routes is Lipozyme RM IM catalyzed desymmetrization of meso-diols **3**. The highest enantiomeric excess (*e.e.* > 98%) and yield (>90%) of the product was achieved with Lipozyme RM IM in Dioxane/Toluene (1:3, v/v) at 35 °C. Furthermore, Lipozyme RM IM showed an excellent operational stability, retaining above 80% of the initial activity after 10 cycles of reaction. *d*-Biotin intermediate lactone **1** was obtained subsequently by Jones oxidation, basic hydrolysis and lactonization.

1. Introduction

d-Biotin (Vitamin H) is a water-soluble vitamin B which is essential to the normal physiological activities of animals and human life. It serves as the prosthetic group of some metabolic enzymes in vivo known as biotin-dependent carboxylases [1–3]. Growth retardation and dysontogenesis will occur when poultry lack biotin. *d*-Biotin has been extensively applied in medicine and health protection, particularly as the feedstuff additive with large commercial requirement. *d*-Biotin intermediate (3a*S*, 6a*R*)-lactone **1** is extremely vital to the synthesis of *d*-biotin [4,5]. Many scientists devoted themselves to the synthesis of (3a*S*, 6a*R*)-lactone **1**, and the asymmetric synthetic routes of (3a*S*, 6a*R*)-lactone **1** have been numerously reported in recent years. Fei Xiong et al. obtained the (3a*S*, 6a*R*)-lactone **1** through a rapid cinchona alkaloid-based sulfonamide-mediated enantioselective alcoholysis of meso-cyclic anhydride [6]. A method through catalytic reactivity in the hydrogenation of a cyclic anhydride to a biotin synthetic intermediate has been investigated using Wilkinson Ru complex by Masahiro Yoshimura et al. [7].

With the development of enzymatic reactions in organic chemistry, more and more chemists pay attention to the study of enzymatic desymmetrization. Due to the ecological aspect of reaction, enzymatic reactions have an advantage status of green chemistry over chemical synthesis [8]. Because of being carried out under mild conditions, enzymatic reactions have the advantage of less side reaction (such as isomerization, racemization, epimerization, and rearrangement of molecules). Enzymatic synthesis of (3a*S*, 6a*R*)-lactone **1** via asymmetric hydrolysis of meso-dicarboxylic esters by pig liver esterase and subsequently following by Grignard reaction was reported [9,10]. Zheng et al. reported that (3a*S*, 6a*R*)-lactone **1** could be efficiently prepared in excellent conversion ratio ($c \geq 40\%$) and enantiomeric purities (*e.e.* $\geq 98\%$) via enantioselective lactonization by the resting cell of *Aspergillus oryzae* WZ007 [11].

Hydrolases have been successfully used for the desymmetrization of different meso or prochiral alcohols, carboxylic acid esters, anhydrides and nitriles [12]. Many authors have demonstrated that enzymatic desymmetrization of the prochiral dimethyl carbonate is a versatile and effective way to obtain the corresponding monoester [13,14]. Two approaches have been employed for the desymmetrization of different meso or prochiral alcohols: acylation of the free alcohol by means of transesterification reaction [15,16], and hydrolysis of an appropriate acyl derivative of alcohol [17,18]. Lipase-catalyzed transesterification may be an efficient method for desymmetrization of meso-diols [19–22].

In this paper, we described a novel synthetic route of (3a*S*, 6a*R*)-lactone **1** by enantioselective transesterification of diols **3** with Lipozyme RM IM and subsequently following Jones oxidation, basic hydrolysis and lactonization (Scheme 1).

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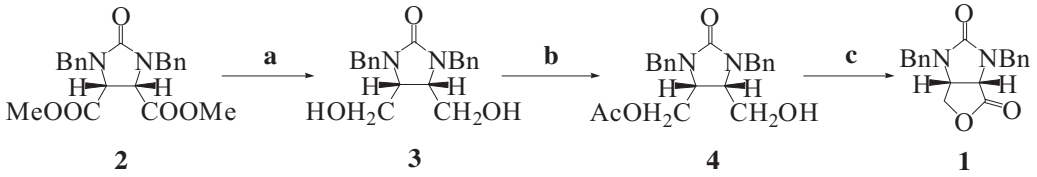


Scheme 1. Synthetic scheme for the synthesis of *d*-biotin intermediate lactone **1**. Reaction conditions: (a) EtOH, $NaBH_4$, 0 °C to r.t. 50 °C, reflux 3 h 96%; (b) Lipozyme RM IM, Dioxane/Toluene (1:3), 6 h, 35 °C, 90%; (c) CrO_3, H_2SO_4, H_2O, acetone, 0 °C, 2 h, then 2 M NaOH, 1 M HCl reflux, 3 h, 65%.

2. Experimental

2.1. General methods

The conversions were determined by Waters 1525 HPLC with a UV detector. Enantiomeric separation of monoacetate **4** was performed using the Chiralcel AD-H column (250 mm × 4.6 mm, 10 μm, Daicel, Japan) and a mixture of hexane and propan-2-ol 80:20 (v/v) as eluant. Flow rate was 0.8 mL/min and retention times for (4*R*, 5*S*)-monoacetate-**4** and (4*S*, 5*R*)-monoacetate-**4** were 19.5 and 23.1 min, respectively. Enantiomeric separation of lactone **1** was performed using the Chiral CD-Ph column (250 mm × 4.6 mm, 10 μm, Shiseido, Japan) and a mixture of acetonitrile and water 60:40 (v/v) as eluant. Flow rate was 0.5 mL/min and retention times for (3a*R*, 6a*S*)-lactone-**1** and (3a*S*, 6a*R*)-lactone-**1** were 12.8 and 14.2 min, respectively. ^{1}H NMR spectra were recorded on a Bruker 500 Hz apparatus (TMS as an internal standard, ^{13}C NMR 125 Hz). Mass spectra were recorded on an Esquire 6000 spectrometer. IR spectra were recorded on Bruker Tensor 27 spectrometer. Optical rotations were determined using an Autopol IV Polarimeter at 25 °C using a cell of 1 dm length.

2.2. Materials

Meso diester **2** and *d*-biotin intermediate lactone **1** was obtained from Zhejiang Shengda Pharmaceutical Co., Ltd. (Zhejiang, China) as a gift. Commercially available organic solvents were treated with 3 Å molecular sieves. All other chemicals employed in this work were obtained from various commercial suppliers.

Novozym 435 (component B of the lipase from *Candida Antarctica* immobilized on macroporous polyacrylate resin), Lipozyme RM IM (*Rhizomucor miehei* immobilized on ionic resin) were supplied by Novozymes A/S (Bagsvaerd, Denmark). Amano lipase AK (*Pseudomonas fluorescens* lipase), Amano lipase A (*Aspergillus niger* lipase), *Rhizopus niveus* lipase, Amano PS IM (lipase from *Burkholderia cepacia* immobilized on diatomaceous earth), and Amano lipase AY (*Candida rugosa* lipase) were obtained from Sigma–Aldrich (shanghai) Trading Co. Ltd. (shanghai, China). Lipase from pig (porcine) pancreas (PPL) was obtained from Aladdin Industrial Co. (shanghai, China).

Jones reagent was prepared as follows: chromium trioxide (26.7 g) was dissolved in sulfuric acid (23 mL) and the resulting mixture was diluted with water to 100 mL.

2.3. Procedure for the synthesis of **3** [23]

A solution of diester **2** (9.53 g, 24.9 mmol) in anhydrous CH_3COOH (200 mL) was cooled to 0 °C and $NaBH_4$ (11.3 g, 30.5 mmol) was carefully added during 10 min. The reaction mixture was stirred for 5 h at room temperature, followed by refluxing for 3 h at 80 °C. And then formic acid was dripped to the above solution slowly until pH fell to about 4–5 at 0 °C. Salts were filtered off through buchner funnel, and the residue was washed with CH_3COOH (3 mL × 20 mL), filtrate was combined and evaporated under reduced pressure, the obtained reaction crude that was purified by recrystallization from *n*-hexane affording 7.63 g of meso-diols **3** as a white solid (94%): ^{1}H NMR($CDCl_3$): δ 7.36–7.27 (m, 10H), 4.92–4.89 (d, 2H, J = 15.40 Hz), 4.14–4.11 (d, 2H, J = 15.45 Hz), 3.84–3.82 (d, 2H, J = 13.00 Hz), 3.75–3.72 (d, 2H, J = 12.90 Hz), 3.51 (s, 2H), 2.37 (s, 2H); ^{13}C NMR($CDCl_3$): δ 161.5, 137.1, 128.8, 127.9, 127.8, 127.6, 57.7, 56.5, 46.6, 45.6; MS (*m*/*z*) 349 $(M + Na)^+$; IR ν_{max} (cm^{-1}): 3442, 3266, 2930, 1670, 1476, 1453, 1359, 1253, 1146, 1054, 1001, 741, 701, 657, 540.

2.4. Procedure for the synthesis of **4**

2.4.1. General procedure for the chemical synthesis of racemic monoacetates **4**

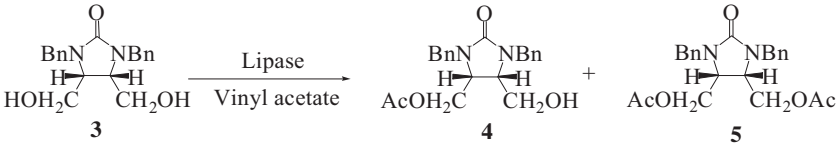
To a solution of diols **3** (2.76 mmol) in dry CH_2Cl_2 (28 mL) were added Et_3N (120 μL, 8.40 mmol) and DMAP (111 mg, 0.90 mmol) under nitrogen atmosphere. After the mixture was stirred for a couple of minutes, Ac_2O (265 μL, 2.8 mmol) was added in portions, and the solution was stirred for an additional 4 h at room temperature. After the solvent was evaporated under reduced pressure, the obtained reaction crude that was purified by flash chromatography (EtOAc/petroleum benzene = 2:1) affording the monoacetate **4** as a colorless oil (54% yield): Rf (EtOAc/petroleum benzene = 2:1) 0.46.

2.4.2. General procedure for the lipase-catalyzed synthesis of monoacetates **4**

A mixture of meso-diols **3** (326 mg, 1 mmol), Lipozyme RM IM (100 mg), and vinyl acetate (555.4 μL, 6 mmol) was stirred in 10 mL Dioxane and Toluene (1:3) at 35 °C for 6 h in water baths shaker. After removal of polymer-supported enzyme by filtration, the filtrate was evaporated under reduced pressure. Then the monoacetate **4** was obtained in a yield of 90% with the high enantiomeric excess (*e.e.* > 98%). $[\alpha]^{25}_D$:−7.2(c 1.0 $CHCl_3$); ^{1}H NMR($CDCl_3$): δ 7.34–7.31 (m, 6H), 7.29–7.26 (m, 4H), 4.87 (d, 2H, J = 15.45 Hz), 4.77 (d, 2H, J = 15.40 Hz), 4.44 (d, 2H), 4.42 (d, 2H), 3.45 (s, 2H), 1.98 (s, 1H), 1.98(s, 3H); ^{13}C NMR($CDCl_3$): δ 170.3, 161.2, 137.4, 137.1, 128.7, 128.6, 128.0, 127.9, 127.8, 127.5, 127.4, 61.6, 58.8, 56.4, 54.1, 54.0, 46.3, 46.2, 21.0, 20.7, 14.1. MS (*m*/*z*) 391 $(M + Na)^+$; IR ν_{max} (cm^{-1}): 3354, 2901, 1744, 1658, 1477, 1451, 1356, 1231, 1044, 760, 739, 700, 457 (Scheme 2).

2.5. Procedure for the synthesis of **5**

To a solution of the corresponding diols **3** (2.76 mmol) in dry CH_2Cl_2 (28 mL) were successively added Et_3N (2.34 mL, 16.62 mmol), DMAP (222 mg, 1.84 mmol), and Ac_2O (1.04 mL, 11.08 mmol) under nitrogen atmosphere. The reaction was stirred at room temperature during 4 h until complete consumption of the starting material, and then the solvent was evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography on silica gel (EtOAc/petroleum benzene = 2:1), yielding the corresponding diacetate **5** as an oil (98%): Rf (EtOAc/petroleum benzene = 2:1) 0.69; ^{1}H NMR($CDCl_3$): δ 7.36–7.28 (m, 10H), 4.87 (d, 2H, J = 15.45 Hz), 4.33–4.32 (m, 2H, J = 5.15 Hz), 4.30–4.29 (m, 2H, J = 5.05 Hz), 4.23 (s, 1H) 4.19 (s, 1H), 3.67 (s, 2H), 2.02 (s, 6H); ^{13}C NMR($CDCl_3$): δ 170.2, 160.6, 137.1,



Scheme 2. Enzymatic desymmetrization of meso-diols **3**.

Table 1
Lipase-catalyzed synthesis of monoacetate **4** using various lipases.

Entry	Enzyme	Conv. [%][a]	Ratio **4**/**5**[b]	*e.e.*(**4**)[%][c]	
				4*R*, 5*S*	4*S*, 5*R*
1	Novozym435	26.22	93:7	33.06	–
2	Amano lipase A	2.29	66:33	24.95	–
3	Amano lipase AK	60.61	96:4	90.83	–
4	Amano PS IM	92.96	98:2	92.62	–
5	Lipozyme RM IM	90.90	94:6	–	90.41
6	PPL	5.74	50:50	–	57.78
7	Amano lipase AY	65.66	98:2	–	86.28

Conditions: diols **3** (25 mmol/L), vinyl acetate (200 mmol/L) and lipases (0.05 g) in CH_2Cl_2 (5 mL) were shaken at 30 °C for 24 h.
[a] Conv. [%] is the conversion of diols **3** was determined by HPLC.
[b] Ratio **4**/**5** is the molar ratio of monoacetate **4** and diacetate **5** and was determined by HPLC.
[c] *e.e.*% of monoacetate **4** was determined by HPLC.

Table 2
The influence of the type of acylating reagent on the selectivity for the enzymatic acylation of **3** by Lipozyme RM IM.

Entry	Acylating reagent	Yield (**5**)[%][a]	Yield (**4**)[%][a]	*e.e.*(**4**)[%][a]
1	Vinyl acetate	5.37	92.94	89.40
2	Ethyl acetate	6.06	12.85	89.34
3	Acetic acid	5.04	4.50	84.24
4	Acetic anhydride	8.52	78.06	77.70
5	Isopropenyl acetate	8.10	24.50	87.63
6	Butyl acetate	20.84	78.96	92.28

Conditions: diols **3** (25 mmol/L), acylating reagent (150 mmol/L) and Lipozyme RM IM (0.05 g) in CH_2Cl_2 (5 mL) were shaken at 30 °C for 24 h.
[a] *e.e.*% and yield of monoacetate **4** and the yield of diacetate **5** was determined by HPLC.

128.6, 128.0, 127.8, 127.5, 60.6, 53.7, 46.2, 20.6, 20.5; MS (*m*/*z*) 433 $(M+Na)^+$; IR ν_{max} (cm^{-1}): 3032, 2998, 2958, 1736, 1685, 1469, 1452, 1369, 1288, 1230, 1037, 922, 767, 700, 606, 565, 483.

2.6. Procedure for the synthesis of *1*

To a solution of monoacetate **4** (0.2 g, 0.5 mmol) in acetone (5.0 mL) was added Jones reagent (0.5 mL) at 0 °C. The mixture was stirred for 2 h, *i*-propanol was added dropwise at the same temperature until discoloration. Then the resulting mixture was filtered through Celite, and the residue was washed with acetone (3 mL × 5 mL). CH_2Cl_2 (5 mL) was added after the filtrate was combined and evaporated under reduced pressure. The mixture was washed with water (3 mL × 5 mL) until colorless, then the organic phase was dried over anhydrous magnesium sulfate and evaporated to afford the carboxylic acid. With the carboxylic acid in hand, prepared THF (2 mL) and 2 M NaOH (5 mL) was added and stirred at 80 °C for 5 h. The THF was evaporated, after which the aqueous phase was extracted with CH_2Cl_2 (3 mL × 5 mL), then 1 M HCl was added to the aqueous phase until pH fell to about 3–4. After 1–3 h stirring at 60 °C, extraction of the aqueous phase with CH_2Cl_2 (3 mL × 5 mL), washing the combined organic phases with brine (10 mL), drying over $MgSO_4$, and evaporation led to an powder: mp 119–121 °C; $[\alpha]^{25}_D$: +59.1 (c 1.0 $CHCl_3$) ^{1}H NMR($CDCl_3$): δ 7.41–7.26 (m, 10H), 5.08 (d, 1H, *J* = 14.8 Hz), 4.67 (d, 1H, *J* = 15.15 Hz), 4.41–4.36 (m, 2H), 4.18 (d, 2H), 3.95 (d, 1H, *J* = 13.60 Hz), 3.75 (d, 1H); ^{13}C NMR($CDCl_3$): δ 172.8, 158.2, 136.0, 135.9, 129.1, 128.9, 128.8, 128.3, 128.1, 127.9, 70.1, 54.4, 52.5, 47.0, 45.2; MS (*m*/*z*) 357 $(M+Na)^+$; IR ν_{max} (cm^{-1}): 3061, 2920, 1775, 1702, 1476, 1445, 1416, 1316, 1237, 1186, 998, 970, 789, 755, 670, 639, 528, 455. The analytical data were consistent with those of the authentic sample of **1**.

3. Results and discussion

3.1. Screening of lipase

Initially, we screened a series of lipases to identify potential catalysts for the title reaction. Seven commercially available lipases were screened, the results were listed in Table 1. Judging from these results, The enzymes could be classified in two groups (Table 1): Entries 1–4: Amano lipase AK and Amano PS IM showed good (60.61%) and high (92.96%) conversions, respectively, to the undesired (4*R*, 5*S*)-monoacetate **4** in high (90.83%, 92.62%) *e.e.* Novozym 435 and Amano lipase A showed lower conversions (<26.22%) and *e.e.* (24.95, 33.06%). Entries 5–7: Lipozyme RM IM and Amano lipase AY showed high conversions (90.90%, 65.66%) to the desired (4*S*, 5*R*)-monoacetate **4** with high *e.e.* (90.41%, 86.28%). PPL showed low (5.74%) conversion with moderate (57.78%) *e.e.* to the desired (4*S*, 5*R*)- monoacetate **4**.

To develop a scalable process for the biocatalytic synthesis of the desired (4*S*, 5*R*)-monoacetate **4**, further studies were conducted following up on the initial screening results. After analyzing several reaction attributes, e.g. yield and *e.e.* of product, reaction rate and cost of enzyme, Lipozyme RM IM was chosen for further development of the enantioselective transesterification of diols **3** to the desired (4*S*, 5*R*)-monoacetate **4**.

3.2. Choice of acyl donor

The obtained results revealed that an acyl group donor has an influence on both selectivity and reaction rate. Six acylating agents were used in enzymatic transesterification, including vinyl acetate, ethyl acetate, acetic acid, acetic anhydride, isopropenyl acetate, and butyl acetate. The acylation reaction of **3** was carried out in CH_2Cl_2 for 24 h with the set of different acylating reagents. The results obtained were summarized in Table 2. As shown in Table 2, the excellent enantioselectivity (*e.e.*92.28%) with a yield (78.96%) was obtained when butyl acetate was used as the acylating agent. But the yield of diacetate **5** reach 20.84% which was undesirable. When other acylating agents were used, the outcome indicated that vinyl acetate was the excellent acylating reagent for the transesterification of diols **3** (Table 2, entry 1) leading to the corresponding monoacetate in high yield (92.94%) and excellent *e.e.* (89.40%) within 24 h.

3.3. Choice of solvent

Since the hydrophobicity of organic solvents can influence the enantioselectivity of lipase-catalyzed reaction, the choice of the solvent used as the reaction medium is very significant in the enzymatic synthesis. A variety of organic solvents were examined. These results are shown in Table 3.

Table 3
Transesterification of diols **3** with vinyl acetate in different organic solvents.

Entry	Solvent	Log P^a	Yield (**5**)[%]	Yield (**4**)[%]	*e.e.*(**4**)[%]
1	Dioxane	−1.10	3.35	47.96	93.69
2	Acetonitrile	−0.33	4.67	70.49	68.51
3	Acetone	−0.23	2.98	72.03	72.66
4	Tetrahydrofuran	0.49	6.56	47.63	76.17
5	Dichloromethane	1.01	5.37	92.94	89.40
6	TBME	1.30	49.26	50.48	80.00
7	Toluene	2.50	13.47	85.66	95.97
8	Cyclohenane	3.40	54.59	44.17	90.71
9	Hexane	3.50	82.61	16.72	60.99
10	Dioxane/Toluene (1:3)	–	12.23	87.33	99.28
11	Dioxane/Toluene (1:1)	–	10.96	86.53	97.23
12	Dioxane/Toluene (3:1)	–	5.89	90.48	95.46

Conditions: diols **3** (25 mmol/L), vinyl acetate (150 mmol/L) and Lipozyme RM IM (0.05 g) in different solvents (5 mL) were shaken at 30 °C for 24 h.

[a] Logarithm of the octanol–water partition coefficient of the solvent [24].

As shown in Table 3, the remarkable influence of organic solvent was observed. Solvents with log P < 1.30 showed low yields of diacetate **5**, while log P > 1.30 showed high yields of diacetate **5**. Dioxane (log P = −1.10) and dichloromethane (log P = 1.01) showed highest *e.e.* of monoacetate **4** and lower yields of diacetate **5** (entry 1), toluene (log P = 2.50) showed a good yield (85.66%) and high *e.e.* (95.97%) of monoacetate **4** (entry 7). When cyclohenane (log P = −3.4) was used as the solvent, we obtained the monoacetate **4** in moderate yield (44.17%) and *e.e.*(90.71%). However the yield of the undesired diacetate **5** reached 54.59% (entry 8). Considering the solubility of diols **3** in different solvent and the results in Table 3, we tried to use mixed solvent to obtain a better result. When a mixture of dioxane and toluene was used as the solvent, we obtained an excellent result (entry 10–12). Excellent enantioselectivity (*e.e.*99.28%) with a high yield (87.33%) was obtained when diols **3** was treated with Lipozyme RM IM in dioxane/toluene (1:3) compared with that in dioxane (yield 47.96%, *e.e.* 93.69%) and in toluene (yield 85.66%, *e.e.* 95.97%) (entry 10). The mixed solvent was chosen for further study.

3.4. Effect of temperature

Temperature has great effect on the activity, selectivity and stability of a enzyme and the thermodynamic equilibrium of a reaction as well [25]. The thermal stability of biocatalysts is always taken as one of the most important criteria for industrial applications [26]. Therefore, the effect of temperature on the enantioselective transesterification of diols **3** was studied. The initial velocity and *e.e.* at different temperatures were shown in Fig. 1. The initial velocity was calculated by the conversion in 2 min, and the conversion was calculated by the concentration decrement of diols 3 by HPLC. Judging from Fig. 1, the initial velocity increased when the reaction temperature was raised since higher temperature accelerates molecular diffusion. However *e.e.* decreased while the reaction temperature raised over 35 °C. This is due to the partial inactivation of the enzyme in organic solvent at high temperature for a long time. Consequently, the optimal temperature was 35 °C because of its highest *e.e.* and acceptable initial velocity.

3.5. Time-courses of Lipozyme RM IM catalyzed desymmetrization of diols **3**

Composition of the reaction mixture during the 24 h of acetylation of diols **3** is shown in Fig. 2. The amount of the diols **3** was rapidly reduced to 9.2% from 100% at the first 1.5 h and was dropt to 0.3% after the reaction for 3 h. That means the substrate diols **3** was complete reaction at the first 3 h. The yield of monoacetate **4** was notably increased to 82.9% during the fist 1.5 h of reaction and

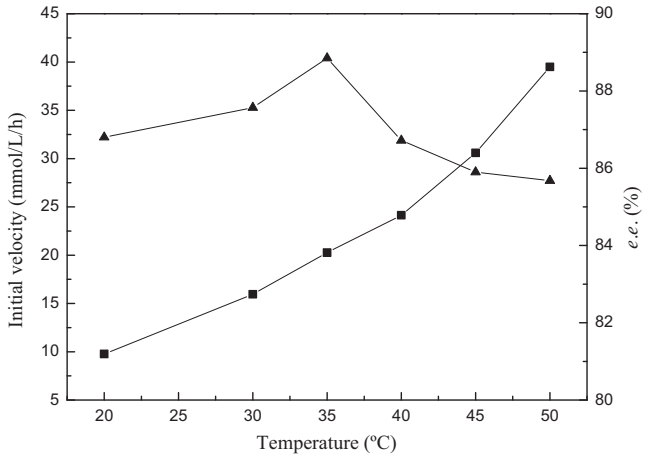


Fig. 1. Effect of temperature on the reaction initial velocity and *e.e.* symbols: initial velocity (■) and *e.e.* (▲) of monoacetate **4**. Conditions: diols **3** (25 mmol/L), vinyl acetate (150 mmol/L) and Lipozyme RM IM (0.05 g) in Dioxane/Toluene (1:3) (5 mL) were shaken at different temperatures. The initial velocity was calculated by the conversion in 2 min.

reached maximum (95.0%) at 3 h. However, it began to decreased after 3 h and dropt to 78% upon progression of the reaction. Furthermore, the production of diacetate **5** was almost not observed in the first 1.5 h but it increased from 0.5% yield at 1.5 h to 5% yield at 3 h and reached 20% at 24 h. Fortunately, the reuse of diacetate **5** was developed by alkali treatment which was done when we obtained *d*-biotin intermediate lactone **1** from monoacetate **4**. On the other hand, the enantiomeric excess of monoacetate **4** was increased from 85% to 99% during the first 6 h, and then slowly decreased to 97% at 24 h.

This is mainly because two steps is contained in the reaction and monoacetates are in principle formed at unequal rates, and also react further at unequal rates in a double step process. The first step is desymmetrization, and the second step is kinetic resolution [27]. Recently it has been demonstrated for several authors that a particular immobilized form of lipases can be control this specificity towards the monoacetylated produced [28,29]. Lowering of the reaction temperature led to an increased enantioselectivity, but the rate ratio of the transformations of diol 3 to monoacetate 4 and of monoacetate 4 to diacetate 5 was not altered by lowering the reaction temperature [30]. Hence monitoring of the reaction

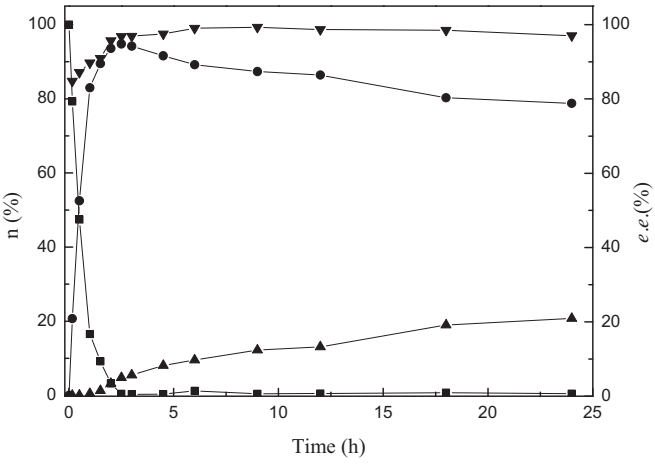


Fig. 2. Time course of enzyme-catalyzed transesterification with the amount of diols **3** (■), monoacetate **4** (●) and diacetate **5** (▲) (*n* [%]) and the *e.e.* of monoacetate **4** (▼). Conditions: diols **3** (25 mmol/L), vinyl acetate (150 mmol/L) and Lipozyme RM IM (0.05 g) in Dioxane/Toluene (1:3) (5 mL) were shaken at 35 °C for 24 h.

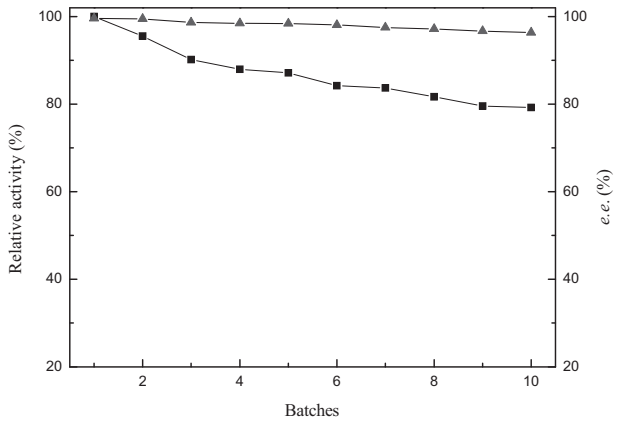


Fig. 3. The reusability of enzyme. symbols: relative activity of the enzyme (■) and the *e.e.* of monoacetate **4** (▲). Conditions: diols **3** (25 mmol/L), vinyl acetate (150 mmol/L) and Lipozyme RM IM (0.05 g) in Dioxane/Toluene (1:3) (5 mL) were shaken at 35 °C.

progress is of utmost importance to determine the reaction in order to obtain the excellent yield and high enantiomeric excess.

3.6. The reusability of enzyme

In general, free enzymes are difficult to recover and reuse, especially in the soluble form as compared to the solid form. Reusability of enzyme-immobilized is an important aspect of the study. We demonstrated the recyclable use of enzyme in this organic solvent system. The immobilized lipase was reused with fresh substrates after each batch. The *e.e* of monoacetate **4** and relative activity of Lipozyme RM IM after recycling several times was given in Fig. 3. The initial activity of freshly prepared immobilized lipase in the first batch was defined as 100% activity. The result showed that the Lipozyme RM IM was highly stable and it retained about 79% of its initial activity after 10 batches. Furthermore, the *e.e.* of monoacetate **4** did not decrease significantly and kept in level of approximate 96% throughout 10 batches. This excellent long-term operational stability of Lipozyme RM IM is an important argument for industrial use of the biocatalysts.

4. Conclusion

In summary, we have developed an efficient methodology for the chemoenzymatic synthesis of *d*-biotin intermediate lactone **1**. The key chiral monoacetate **4** was obtained by Lipozyme RM IM catalyzed enantioselective transesterification of diols **3**. The preferable solvent (dioxane/toluene (1:3)) and reaction condition (35 °C, 200 rpm) were determined. Lipozyme RM IM was found to be effective for its stability and reusability in our experiment. Finally, the target product *d*-biotin intermediate lactone **1** was obtained in high *e.e.* (>98%) and excellent yield (65%) from the chiral monoacetate **4** by Jones oxidation, basic hydrolysis and lactonization. Currently, we are investigating its implementation in the practical large-scale preparation of *d*-biotin.

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