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Note

Efficient Preparation of (*R*)-3-Hydroxypentanenitrile with High Enantiomeric Excess by Enzymatic Reduction with Subsequent Enhancement of the Optical Purity by Lipase-Catalyzed Ester Hydrolysis

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An efficient chemo-enzymatic procedure for the synthesis of (*R*)-3-hydroxypentanenitrile (1) with over 99% enantiomeric excess using two enzymatic reactions was successfully established. Initial enantioselective enzymatic reduction of 3-oxopentanenitrile with reductase S1 gave (*R*)-1 with an 81.5% ee which was then converted to (*R*)-1-(cyanomethyl) propyl *n*-butyrate (3b). Subsequent lipase-catalyzed enantioselective hydrolysis of 3b gave (*R*)-1 in a high yield with over 99% ee.

Key words: (*R*)-3-hydroxypentanenitrile; enantioselective enzymatic hydrolysis; enantioselective enzymatic reduction

Chiral β-hydroxynitriles are very common intermediates in organic synthesis. (*R*)-3-Hydroxypentanenitrile (**1**) is an important intermediate in the synthesis of an immunosuppressive inosine 5′-monophosphate dehydrogenase inhibitor,[1] in which (*R*)-**1** with a high enantiomeric excess (>99% ee) is required for the synthesis. Only one preparation of (*R*)-**1** with an ee in excess of 99% has been reported, involving the *Pseudomonas cepacia* lipase-catalyzed kinetic resolution of (±)-1-(cyanomethyl) propyl acetate (**3a**) in the presence of a thiacrown ether.[2] This method, however, is unsuitable for the industrial-scale production of (*R*)-**1**, because of the requirement for an expensive thiacrown ether reagent and difficulties associated with the separation of the (*R*)-**1** product from the remaining substrate **3a** which can only be achieved by column chromatography. Furthermore, the yield of (*R*)-**1** from this process was low (49%) because of the optical resolution. With this in mind, we report here an efficient synthetic procedure for (*R*)-**1** which is amenable to industrial-scale production.

Many procedures have been developed for the preparation of optically active alcohols.[3] One of the most convenient methods involves the enzyme-assisted asymmetric reduction of carbonyl compounds.[4,5] In many cases, enzymatic reduction of carbonyl compounds, under the appropriate conditions, affords a chiral alcohol with high optical purity. It is convenient that 3-oxopentanenitrile (**2**) could be easily prepared from ethyl propionate and acetonitrile.[6] We also had a collection of various reductases and alcohol dehydrogenases that could be coupled with a coenzyme-regeneration system containing glucose dehydrogenase,[4,5] these tools being applied in the current study.

The yield, absolute configuration and optical purity of **1** obtained during this study were determined by gas chromatography under the following conditions: A Chiraldex G-TA (ASTEC, USA) column (0.25 mm i.d. × 30 m) was used with He carrier gas at 150 kPa, and respective injection and column temperatures of 200 and 115 °C. An FID detector was used for visualization at 200 °C. The respective retention times (t_R) for (*R*)-**1** and (*S*)-**1** were 7.5 and 8.3 min. Several enzymes produced **1** with the (*R*) configuration, with carbonyl reductase S1[7] showing the highest level of (*R*)-enantioselectivity, although the enantiomeric excess of (*R*)-**1** obtained was only 81.5% ee. To enhance the enantiomeric excess of (*R*)-**1** obtained by enzymatic reduction to greater than 99% ee, we investigated the hydrolysis of acylated (*R*)-**1** with a hydrolase (Fig. 1A).

We also simultaneously investigated the development of a practical method for separating the remaining substrate, acylated **1** (**3**), from product (*R*)-**1** following the hydrolysis reaction which did not involve column chromatography. Focusing on the differences in the lipophilicity of the two compounds, separation by extraction with *n*-hexane was undertaken. A 10-mg sample of racemic **3**, (±)-1-(cyanomethyl)propyl acetate (**3a**) or (±)-1-(cyanomethyl)propyl butyrate (**3b**) which had been prepared from (±)-**1** and acyl chloride according to the reported procedures,[2] 100 mg of (*R*)-**1** prepared as already described and 1.0 mL of a 0.5 M potassium phosphate buffer (pH 7.0) were mixed and extracted with *n*-hexane (3 × 0.3 mL). Extraction with *n*-hexane recovered (±)-**3a** in a 45% yield, whereas (±)-**3b** was recovered in an over 99% yield. More water-soluble **1** was confined to the aqueous phase. Thus, **3b** could be removed in a high yield and separated from **1** by extracting with *n*-hexane, and butylated **1** (**3b**) was selected as the substrate for the hydrolysis reaction.

With the exception of **3a**,[2] the enzymatic enantioselective hydrolysis of acylated **1** (**3**) has not previously been reported. Hence, fifteen commercially available hydrolases (lipases and proteases) were tested for their

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Abbreviation: ee, enantiomeric excess

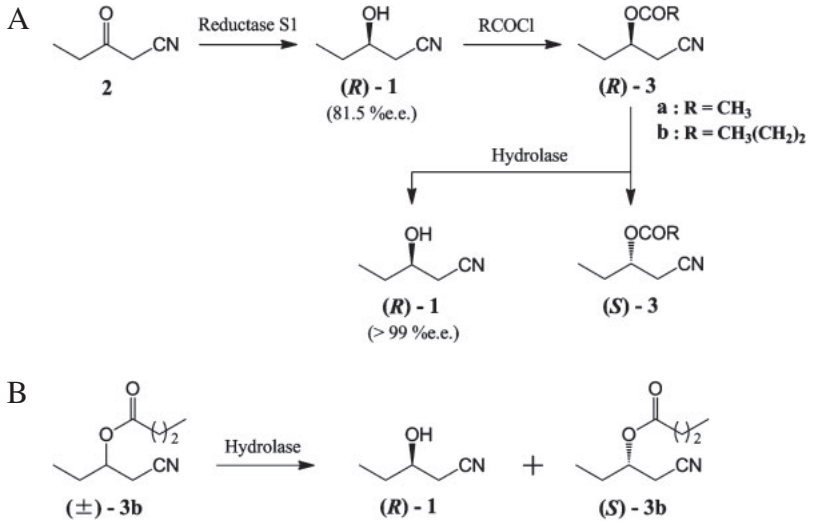

Fig. 1. Synthesis of (*R*)-3-Hydroxypentanenitrile.
A, Synthesis of (*R*)-3-hydroxypentanenitrile with over 99% ee. B, Synthesis of (*R*)-3-hydroxypentanenitrile by enantioselective hydrolysis of racemic 1-(cyanomethyl) propyl *n*-butyrate.

Table 1. Hydrolysis of (±)-1-(Cyanomethyl) propyl Butyrate (**3b**) to 3-Hydroxypentanenitrile (**1**) with Different Hydrolases

Hydrolase (origin)[a]		Reaction conditions[c]	Reaction time (h)	Yield (%)[c]	Config.[c]	% ee[c]	*E* value
Lipase SP398	(*Candida* sp.)	A	20	27	*R*	82	14
Lipase M	(*Mucor javanicus*)	A	20	36	*R*	94	53
Lipase SP388	(*Rhizomucor miehei*)	A	20	52	*R*	82	27
Thermolysin	(*Bacillus thermoproteolyticus*)	A	20	0			
α-Chymotrypsin	(Bovine Pancreas)	A	20	42	*S*	35	0
Lipase AL	(*Achromobacter* sp.)	B	18	53	*R*	50	5
Lipase PL	(*Alcaligenes* sp.)	B	18	12	*R*	68	6
Novozyme 435	(*Candida antarctica*)	B	18	65	*R*	54	18
Lipase MY	(*Candida cylindracea*)	B	18	32	*R*	6	1
Lipase AYS	(*Candida rugosa*)	B	18	37	*R*	5	1
Lipase AK	(*Pseudomonas fluorescence*)	B	18	36	*R*	91	35
TOYOBO LIP	(*Pseudomonas* sp.)	B	18	64	*R*	49	8
Lipase AH	(*Burkholderia cepacia*)	C	1.5	43	*R*	77	14
Lipase PS	(*Burkholderia cepacia*)	C	4	47	*R*	93	73
Lipase SL	(*Pseudomonas cepacia*)	C	1.3	37	*R*	76	12

[a]Lipase SP398, Lipase SP388 and Novozyme 435 were purchased from Novozymes, Denmark. Lipase M, Lipase AYS, Lipase AK, Lipase AH and Lipase PS were purchased from Amano Enzyme, Japan. Lipase AL, Lipase PL, Lipase MY and Lipase SL were purchased from Meito Sangyo, Japan. TOYOBO LIP was purchased from Toyobo, Japan. Thermolysin and α-Chymotrypsin were purchased from Nacalai Tesque, Japan.
[b]Ten milligrams of (±)-**3b**, 5 mg of hydrolase and 0.5 mL of 0.5 M potassium phosphate buffer (pH 7.0) were mixed in a test tube and then stirred at 30 °C (Reaction conditions A). Fifty milligrams of (±)-**3b**, 0.25 mg of enzyme and 0.5 mL of 0.5 M potassium phosphate buffer (pH 7.0) were mixed in a test tube and then stirred at 30 °C (Reaction conditions B). Fifty milligrams of (±)-**3b**, 1 mg of enzyme and 0.5 mL of 0.5 M potassium phosphate buffer (pH 7.0) were mixed in a test tube and then stirred at 30 °C (Reaction conditions C).
[c]Yield, absolute configuration and ee value of **1** were determined by gas chromatography analysis under the following conditions. A Chiraldex G-TA (ASTEC, USA) column (0.25 mm i.d. × 30 m) with He carrier gas at 150 kPa was used with injection and column temperatures of 200 and 115 °C, respectively. Detection by FID was conducted at 200 °C. The retention times (t_R) of (*R*)-**1** and (*S*)-**1** were 7.5 and 8.3 min, respectively.

ability to enantioselectivity hydrolyze (±)-**3b** (Fig. 1B). Mixtures of (±)-**3b**, a hydrolase and a 0.5 M potassium phosphate buffer (pH 7.0) were agitated at 30 °C. The yield, absolute configuration and optical purity of obtained **1** were determined upon completion of the reaction (Table 1). All of the lipases tested showed (*R*)-selectivity, with Lipase PS showing the highest *E* value[8] of 73. Itoh *et al.* have reported that Lipase PS hydrolyzed (±)-**3a** to (*R*)-**1** and (*S*)-**3a** with enantioselectivity and an *E* value of 53.[2] Hence, **3b** was a more suitable substrate than **3a** for enantioselective hydrolysis when using Lipase PS.

(*R*)-**1** of over 99% ee was prepared from inexpensive materials by using two enantioselective enzymatic reactions. Two hundred grams of **2** was prepared from ethyl propionate and acetonitrile (71.0% yield).[6] Three liters of a culture broth of *E. coli* HB101 carrying pSNTS1G, co-overproducing the transformant of carbonyl reductase S1 and glucose dehydrogenase for NADPH regeneration,[9] 45 g of **2**, 300 g of glucose and 100 mg of $NADP^+$ were mixed and then stirred at 30 °C for 22 h. The pH value of the reaction mixture was maintained at 6.5 by the automatic addition of 6 M NaOH. A 45-g charge of **2** was given 2 h and 4 h after the start of the reaction. The reaction mixture was extracted twice with ethyl acetate upon completion of the reaction. The organic layers were combined and the solvent removed *in vacuo* to give (*R*)-**1** with an 81.5% ee

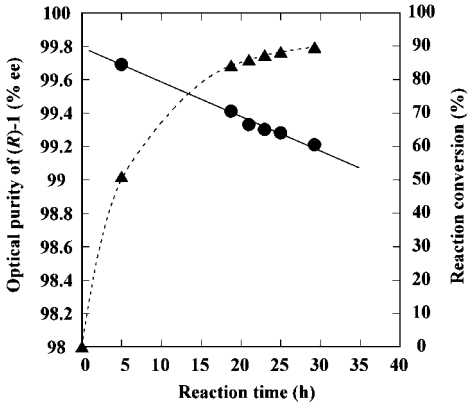

Fig. 2. Enzymatic Hydrolysis of (*R*)-**3b** (81.5% ee) to (*R*)-**1** by Using Lipase PS.

Filled circles indicate the optical purity of (*R*)-**1**; filled triangles indicate the reaction conversion.

(126.0 g, 91.4% yield). A mixture of (*R*)-**1** (119.7 g), 114.6 g of pyridine, 150 mg of *N*,*N*-dimethyl-4-aminopyridine and 300 mL of *tert*-butyl methyl ether was agitated at 0 °C. *n*-Butyryl chloride (167.2 g) was then added to the solution, and the resulting mixture was stirred overnight at room temperature. The reaction mixture was then extracted with ethyl acetate, and the organic layer was collected, dried with anhydrous Na_2SO_4 and the solvent removed *in vacuo* to give crude (*R*)-**3b**. All of this (*R*)-**3b** and 1.25 g of Lipase PS were added to 2.5 L of a 0.5 M potassium phosphate buffer (pH 7.0), and the resulting mixture was stirred at 30 °C for 29 h. Time-course plots of the yield and optical purity for (*R*)-**1** are shown in Fig. 2. Following this reaction, remaining **3b** was removed by extracting with *n*-hexane (4 × 3.0 L). The pH value of the remaining aqueous phase was adjusted to 8.5 by using aqueous NaOH, and (*R*)-**1** was then extracted with ethyl acetate. The combined organic layers were then washed with aqueous $NaHCO_3$ and dried with anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the resulting residue purified by distillation to give (*R*)-**1** with 99.2% ee as a colorless oil (89.8 g, 75.1% yield from (*R*)-**1** obtained by the enzymatic reduction of **2**); NMR δ_H ($CDCl_3$): 1.00 (3H, t, $J = 7.6$ Hz, $-CH_2-CH_3$), 1.64 (2H, dq, $J = 7.3$, 7.3 Hz, $-CH_2-CH_3$), 2.38 (1H, bs, –O*H*), 2.50 (1H, dd, $J = 16.6$, 6.4 Hz, –C*H*2–CN), 2.58 (1H, dd, $J = 16.8$, 4.6 Hz, –C*H*2–CN), 3.83–3.93 (1H, m, –C*H*OH–). The total yield of (*R*)-**1** from **2** was 68.6%.

In summary, we successfully prepared (*R*)-**1** of high enantiomeric purity (>99% ee) and good yield by using two kinds of enantioselective enzymatic reactions. The perspective of enantiomeric ratio (*E* value) for the hydrolysis of **3** indicated **3b** to be a better substrate for Lipase PS than **3a**. In addition, **3b** was easily separated from desired product (*R*)-**1** by extracting with *n*-hexane following the enzymatic hydrolysis. It is very important for industrial-scale production to establish simple methods for removing the impurities without using column chromatography. Selecting **3b** as a substrate for the enzymatic hydrolysis enabled a chemo-enzymatic procedure suitable for the industrial-scale production of (*R*)-**1** with over 99% ee according to Fig. 1A to be successfully established.

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