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An Efficient Continuous Kinetic Resolution of Racemic 2-aminobutanol over Immobilized Penicillin G Acylase

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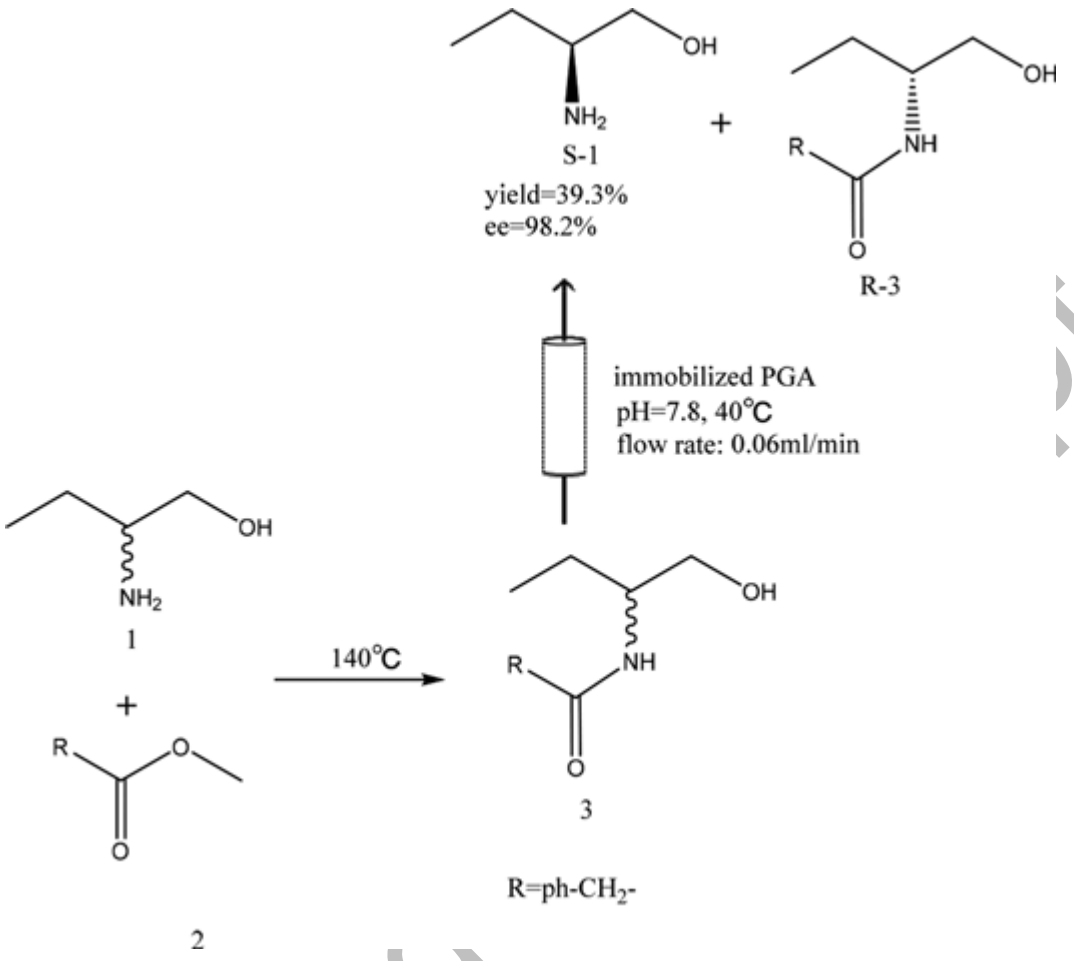
Abstract

In this paper, an efficient method was established for continuous kinetic resolution of racemic 2-aminobutanol by selective hydrolysis of N-phenylacetyl (±)-2-aminobutanol over immobilized penicillin G acylase (PGA) in a fixed-bed reactor. Several N-acylated derivatives of 2-aminobutanol were screened in batch experiments, it was found that the hydrolysis of N-phenylacetyl (±)-2-aminobutanol proceeded smoothly in the presence of immobilized penicillin G acylase with satisfied enantioselectivity. Thus, the reaction parameters were optimized in a fixed-bed reactor. Under the optimized conditions, 39.3% conversion of N-phenylacetyl (±)-2-aminobutanol and 98.2% ee value of S-2-aminobutanol were obtained. This fixed-bed system was operated continuously for 40 hours without significant decrease of enzyme activity. It has been demonstrated to be more efficient compared to the batch experiments.



KEYWORDS: kinetic resolution; 2-aminobutanol; continuous; penicillin G acylase; hydrolysis

INTRODUCTION

Optically pure amines are widely used as resolving reagents, chiral ligands and chiral synthetic building blocks for pharmaceutical and agrochemical industries[1,2]. Since the ever growing need of pharmaceutical and fine chemical industries for optically pure amines, the approaches of preparing optically pure amines have been continuously developed for the last two decades, including transition metal or organocatalyzed

asymmetric synthesis of chiral amines[3,4,5], chemo-enzymatic synthesis, chemical resolution through the formation of a diastereomeric salt[6], and enzymatic kinetic or dynamic resolution[7,8,9]. Among the above mentioned methods, enzymatic resolution has been successfully applied in numerous stereoselective biotransformations by virtue of its excellent regio- and enantioselectivity[10,11,12].

Recently, continuous-flow process represents an attractive trend applied in biotechnology and chemistry[13]. Compared with batch process, it presents many advantages, such as precise control over reaction conditions, including time, temperature and pressure; improvement of productivity; safety; ease of product isolation; possibility of automation and ease of scale-up[14]. Therefore, a few studies employed continuous-flow conditions to realize the kinetic resolution of racemic compounds[15,16,17].

S-2-aminobutanol is the key intermediate of ethambutol, which is an antibiotic for the treatment of tuberculosis[7,18], and also used as chiral auxiliaries[19]. To the best of our knowledge, few example of enzymatic resolution of racemic 2-aminobutanol under continuous-flow conditions has been reported in the literatures. Thus, the continuous kinetic resolution of racemic 2-aminobutanol catalyzed by penicillin G acylase from *Bacillus megaterium* immobilized on polymethacrylate resin in a fixed-bed reactor is summarized and reported here. Firstly, several N-acylated derivatives of racemic 2-aminobutanol were synthesized by the nucleophilic substitution of carboxylic esters with

racemic 2-amino-butanol. Their hydrolysis behavior were then evaluated in the presence of penicillin G acylase based on the principle of enantioselective hydrolysis of N-acylated derivatives of racemic 2-aminobutanol over penicillin G acylase. Finally, it was found that the S-enantiomer of N-acylated 2-aminobutaonl is selectively hydrolyzed to yield S-2-aminobutanol over immobilized PGA.

RESULTS AND DISCUSSION

Initially, racemic 2-aminobutanol was acylated with several selected carboxylic esters (see ESI † for further detail). Then, the hydrolysis performance of the obtained N-acylated derivatives of 2-aminobutanol in the presence of immobilized PGA were examined and screened in batch experiments, the obtained results were summarized in . It was found that the hydrolysis of N-benzoyl-2-aminobutanol (**3a**) was not detected while the hydrolysis of N-phenylacetyl-2-aminobutanols proceeded smoothly over the immobilized PGA, this is possibly attributed to the conjugation of benzene ring and carbonyl group, decreasing the activity of N-benzoyl-2-aminobutanol. So, N-benzoyl-2-aminobutanol can't serve as the resolution substrates of 2-aminobutanol.

As described in Table Ⅰ, S-2-aminobutanol was efficiently obtained in 99% ee at the 33.8% conversion of **3b** (entry 2), but with the increase of **3b** conversion to 50.5%, the ee value of S-2-aminobutanol dropped rapidly to 87.3%. It was found that excess hydrolysis of **3b** could lead to an obvious decrease of enantioselectivity. Then other derivatives were

examined (entry 3-4), it was found that the substituents at benzene ring in substrates also had obvious influence on the reaction activity and enantioselectivity, both of **3c** and **3d** displayed less reaction activity than **3b** over immobilized PGA, but **3c** presented better enantioselectivity (E=96.6) than **3b** (E=44.0) and **3d** (E=17.2). However, **3c** exhibits poor solubility in water, so **3b** was more suitable to be chosen as a resolution substrate for S-2-aminobutanol.

In order to improve the productivity of S-2-aminobutanol and simplify the process, a continuous system was established with fixed-bed reactor. Thus, 0.3g immobilized PGA mixed with 3.0g silica sand was packed into a 2ml glass column, which was heated by a water-bath, it was then fed with 0.1mol/L **3b** in ammonia solution (pH=7.8). The effects of temperature on the kinetic resolution of racemic **3b** were examined in a range from 20℃ to 60℃ in the fixed-bed reactor at 0.04ml/min flow rate. The conversions of **3b** at different temperatures are shown in Figure 1. When the temperature increased from 20℃ to 40℃, the conversion of **3b** rapidly increased to 40%. With the temperature going on increasing, the conversion of **3b** didn't increase obviously, mainly because the higher temperature can destroy the enzymes. with consideration of the conversion of **3b** and the lifetime of immobilized PGA, 40℃ was chosen for the reaction temperature in further experiments.

Next, the effect of flow rate on the continuous kinetic resolution of racemic 2-aminobutanol over immobilized PGA was investigated and the results are shown in Table

Ⅱ. It was found that with the decrease in flow rate from 0.12ml/min to 0.02ml/min, the conversion of **3b** increased from 28.1% to 50.6%. The ee value of S-2-aminobutanol kept more than 99% with the flow rate ranging from 0.12ml/min to 0.08ml/min, but it began to decrease from 99% to 86.8%, when the flow rate went on decreasing to 0.02ml/min. There is no doubt that the immobilized PGA could hydrolyze S-**3b** much faster than R-**3b**. But when majority of S-**3b** was hydrolyzed, the concentration of S-**3b** decreased obviously, this would give R-**3b** more chances to combine with the active site of the immobilized PGA, then R-**3b** was hydrolyzed faster than before. Excess hydrolysis would decrease the ee value of S-2-aminobutanol. So a suitable flow rate of 0.06ml/ml was chosen for the kinetic resolution of racemic 2-aminobutanol in a fixed-bed reactor. Thus, under the optimized resolution conditions, 39.3% conversion of **3b** and 98.2% ee value of S-2-aminobutanol were realized.

In addition, the service time of the immobilized PGA was also evaluated during the kinetic resolution for S-2-aminobutanol using **3b** as the substrate at 40 °C and 0.06ml/min flow rate. As shown in Figure 2, the immobilized PGA catalytic activity didn't decrease obviously after 40 hours of operation. Samples were taken every 3 hours and analyzed by HPLC.

Compared with the reported results, this continuous-flow catalytic system realized a faster resolution of racemic 2-aminobutanol at the same conversion and better robustness. It is

mainly attributed to that the continuous-flow catalytic system is in favour of the absorption- desorption balance of product on PGA. What is more, the continuous-flow catalytic system can also avoid the mechanical damage of PGA caused by vigorously stirring in batch experiments.

CONCLUSION

In summary, an efficient kinetic resolution of racemic 2-aminobutanol was successfully realized by the selective hydrolysis of N-phenylacetyl-2-aminobutanol over PGA with excellent enantioselectivity in a fixed-bed reactor. The resolution parameters, such as temperature and flow rate, were optimized in a fixed-bed reactor. Under the optimized conditions this continuous-flow procedure led to 39.3% conversion of N-phenylacetyl-2-aminobutanol and 98.2% ee value of S-2-aminobutanol at 40℃ and 0.06 mL/min, and no obvious decrease of immobilized PGA catalytic activity was detected in 40h.

EXPERIMENTAL

Penicillin G acylase from Bacillus megaterium immobilized on polymethacrylate was purchased from NovoCata (200U/g). all the other chemicals were purchased from Tianjin Jiangtian Chemical Co., Ltd., Tianjin, China. NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chiral HPLC was performed using a SP-086 Series HPLC (Baseline) with a UV detector and Daicel Chiralpak AS-H column 5×250 mm.

Preparation Of N-Acylated Derivative Of Racemic 2-Aminobutanol -General Process

The carboxylic esters (0.08mol) were respectively heated with racemic 2-aminobutanol (0.08 mol) in a 50ml round bottomed flask at 140°C. The reaction was followed by TLC. After the reaction, the residue was then recrystallised from chloroform–hexane.

Batch Experiment – General Procedure

The racemic substrate **3b** (0.08g, 0.4mmol) was dissolved in 4ml water by warming, the pH was adjusted to 7.8 with ammonia and immobilized PGA (0.04g) was added. The reaction mixture was stirred at 100 rpm with an overhead stirrer at 40°C. The conversion during the hydrolysis was followed by HPLC.

Fixed-Bed Experiment- General Procedure

Immobilized PGA(0.3 g) mixed with silica sand(3.0g) was packed into a glass column with a water-bath for maintaining temperature at 40 °C. it was then fed with 0.1mol/L **3b** in ammonia solution (pH=7.8) at various flow rates (0.12, 0.10, 0.08, 0.06, 0.04 and 0.02 mL/min),0.5mL of samples were collected for HPLC analysis.

N-Phenylacetyl-(±)-2-Aminobutanol (3b)

white powder; mp 62°C;IR (CHCl3): 3468, 1616 cm−1 ;1H NMR (CD3OD, 400MHz): δ= 0.88 (t, 3H), 1.3–1.7 (m, 2H), 2.7 (br s,1H), 3.5–3.9 (m, 5H), 5.5 (br s, 1H), 7.4 (m,

5H).13C NMR (CD3OD, 400 MHz):δ= 10.44, 23.61, 43.62, 57.05, 63.58,127.56, 129.14, 129.95, 135.60, 171.30. MS: m/z calcd for C12H17NO2+: 207.1320; found: 207.2715 [M + H] +.

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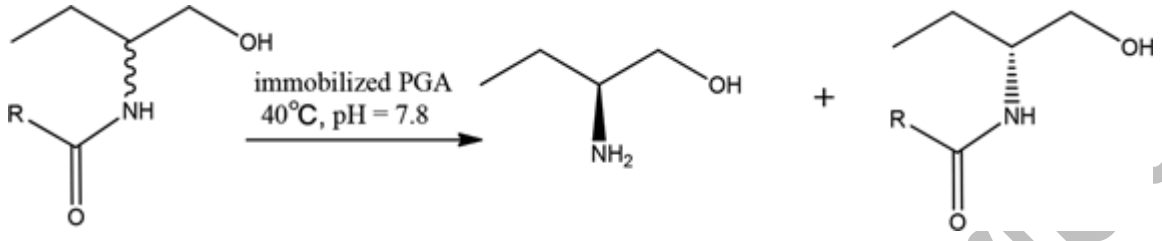
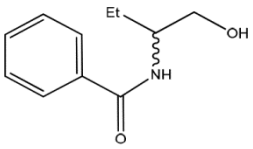
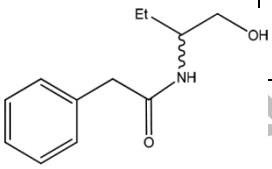
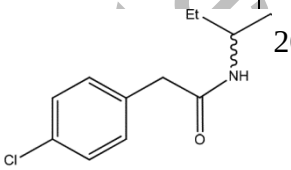
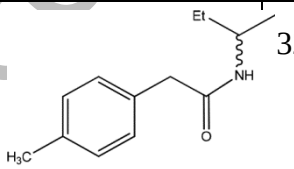
Table I. Screening of the N-acylated derivatives for the kinetic resolution for S-2-aminobutanol[a]



Entry	N-acylated derivative	Time(h)	ee_s[b](%)	ee_p[c](%)	conversion[d] (%)	E[d]
1	 **3a**	20	-	-	-	-
2	 **3b**	6	50.6	99.0	33.8	>200
		10	89.0	87.3	50.5	44.0
3	 **3c**	26	96.1	92.2	51.0	96.6
4	 **3d**	32	80.2	75.4	51.5	17.2

[a]Reaction conditions: 0.4mmol substrate in 4ml of ammonia solution (pH=7.8) in the presence of 0.04g immobilized PGA at 40℃ with 180 rpm.

[b]The ee value of the substrate was determined by chiral HPLC on a chiralpak AS-H column.

[c]The ee value of the product was determined by chiral HPLC on a chiralpak AS-H column after it was acylated by benzoyl chloride.

[d]The conversion was calculated by $C=ee_s/(ee_s+ee_p)$, Enantiomeric ratios (E) were calculated by $E=\ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$.

Table II. Effects of flow rate (residence time) on the kinetic resolution for S-2-aminobutanol by immobilized PGA in a fixed-bed reactor[a]

Entry	Flow rate(residence time)	ee_s(%)	ee_p(%)	conversion(%)	E
1	0.12ml/min(6.7min)	38.7	>99	28.1	＞200
2	0.10ml/min(8min)	48.9	>99	33.0	＞200
3	0.08ml/min(10min)	57.4	>99	36.6	＞200
4	0.06ml/min(13.3min)	63.6	98.2	39.3	＞200
5	0.04ml/min(20min)	73.9	94.3	43.9	75.7
6	0.02ml/min(40min)	89	86.8	50.6	42.0

[a]Reaction conditions: a fixed-bed reactor packed with 0.3g immobilized PGA(mixed with 3.0g silica sand), 0.1mol/L **3b** in ammonia solution(pH=7.8), 40℃.

[b]The ee of the substrate was determined by chiral HPLC on a chiralpak AS-H column.

[c]The ee of the product was determined by chiral HPLC after acylated by benzoyl chloride.

[d]The conversion was calculated by: $C=ee_s/(ee_s+ee_p)$, Enantiomeric ratios (E) were calculated by $E=\ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$.

Figure 1. Effects of temperature on the kinetic resolution for S-2-aminobutanol by immobilized PGA in a fixed-bed reactor

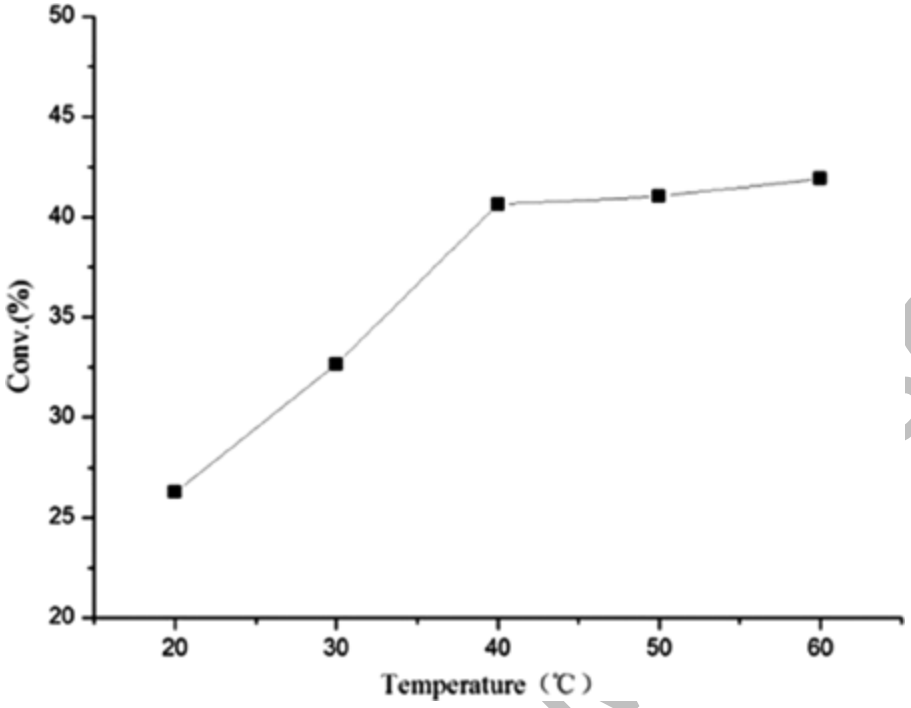


Figure 2. Recyclability of the continuous-flow system