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Enantioselective Acylation of β-Aminoesters Using Penicillin G Acylase in Organic Solvents

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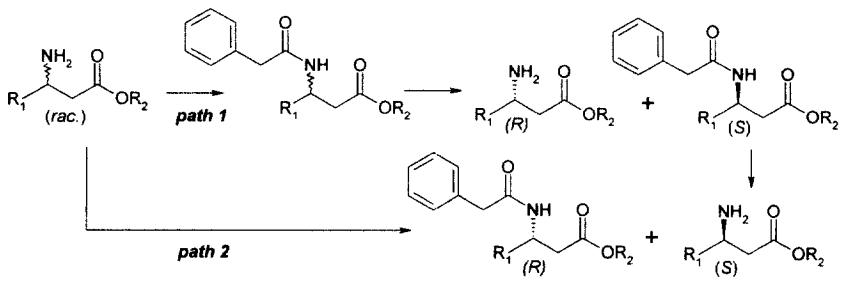
Abstract: The resolution of racemic β-aminoesters has been achieved through selective acylation catalyzed by Penicillin G Acylase (ChiroCLEC™-EC). The method has been optimized using three different phenylacetyl donors, and the effect of solvents on the rate of reaction is described. The efficiency of our method is illustrated by the synthesis of five different β-aminoesters with high enantiomeric purities.

β-Aminoacids are of immense interest as intermediates in organic and medicinal chemistry. Their enantioselective preparation emerged as a challenging task and has been the subject of several reviews.[1a-c] Although elegant catalytic asymmetric methods have been described recently in the literature,[2a-b] most of them are tedious and not amenable for large scale purposes. As racemic β-aminoacids are accessible in one step from the reaction of readily available aldehydes with malonic acid and ammonium acetate, resolution still appears more practical on a preparative scale. The recently reported Penicillin G Acylase (PGA) catalyzed hydrolysis of phenylacetamido derivatives of racemic β-aminoesters leading to pure (*R*)-β-aminoesters (*path 1*)[3] is an example in this context.



However, the application of this methodology to synthesize the (*S*)-enantiomers requires one additional step and is less attractive. We therefore explored the resolution of β-aminoesters through enantioselective acylation (*path 2*) with the possibility of isolating the pure (*S*)-enantiomers in fewer steps. Our studies were focussed on using cross-linked ChiroCLEC™-EC[4], and the results are summarized in *Table 1*.

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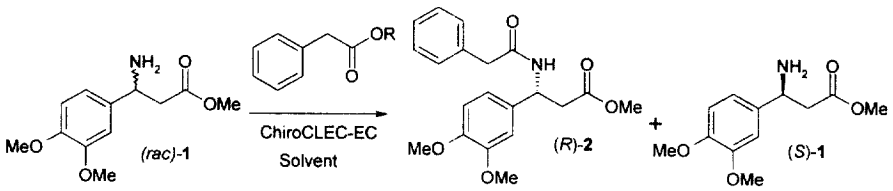
Table 1: Kinetic resolution of racemic methyl 3-amino-3-(3,4-dimethoxyphenyl)propionate.



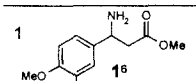
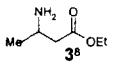
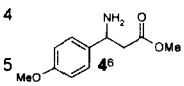
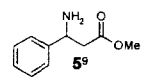
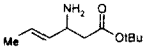
entry	R (eq.)	solvent	conditions	**2** (% conv.)[a]	**1**, yield (% *ee*)[b]
1	H (1)	H_2O (pH 6)	rt, 70 h	29	
2	H (2)	H_2O (pH 5.7)	rt, >120 h	40	55 (65)
3	H (1)	EtOAc/H_2O (1/1)	rt, 29 h	48	
4	CH_2CF_3 (1)	EtOAc/H_2O (1/1)	rt, 29 h	36	
5	Me (0.9)	Dioxane	rt, 6 days	0	
6	Me (1.5)	CH_3CN	rt, 6 days	0	
7	Me (1)	EtOAc	rt, 6 days	0	
8		neat $PhCH_2CO_2Me$	rt, 6 days	0	
9	H (1)	EtOAc/H_2O (98/2)	rt, 48 h	36	
10	CH_2CF_3 (1)	EtOAc/H_2O (98/2)	rt, 29 h	55	
11	Me (1)	EtOAc/H_2O (98/2)	rt, 18 h	52	46 (>95)

a) determined by HPLC based on the disappearance of starting material; b) determined by ^1H NMR using (*R*)-(+)-BPTPA[5] as a chiral shift reagent.

The reaction conditions were optimized using racemic methyl 3-amino-3-(3,4-dimethoxyphenyl)propionate **1**[6] as a substrate and three different phenylacetyl donors. The rate of acylation was slow using $PhCH_2CO_2H$ (1 eq) in water at pH 6. A conversion of 29% was observed after 70 h (*entry 1*) and the enantiomer (*S*)-**1** was obtained with only 65% *ee* after 120 h (*entry 2*). Using a biphasic mixture of ethyl acetate/water (1/1), the acylation rate was considerably increased (*entry 3*), and a 48% conversion was obtained after 29 h. Comparable results were obtained using $PhCH_2CO_2CH_2CF_3$ as the acylating agent under the same conditions (*entry 4*). Whereas all our attempts to run the reaction in dry organic solvents or neat $PhCH_2CO_2Me$ failed (*entries 5-8*), a dramatic change was observed when a 1-2% of water in EtOAc was used. Although the rate of reaction was still slow using $PhCH_2CO_2H$ (*entry 9*), acylation with $PhCH_2CO_2Me$ and $PhCH_2CO_2CH_2CF_3$ appeared significantly faster. HPLC monitoring showed in both cases a 50% of conversion after only 7 h. At 52% conversion, the desired (*S*)-**1** was recovered in 46% yield and >95% *ee (entry 11)*. It is worth noting that although ChiroCLEC™-EC is known to be stable in organic solvents such as EtOAc or toluene, there is no precedent to our knowledge for using this catalyst in organic solvents for amidations. As in the case of lipase or protease catalyzed reactions,[7] a trace of water appears to be enough for triggering the catalytic activity of cross-linked PGA.

The above conditions were applied for the resolution of various racemic β-aminoesters (*Table 2*). This method is suitable for the synthesis of ethyl 3-aminobutyrate, (*R*)-**3**, and methyl-3-amino-3-*p*-methoxyphenylpropionate, (*S*)-**4**, in good enantiomeric purities (94% and 82% respectively; *entries 3 and 4*).

Table 2: Kinetic resolution of racemic β-aminoesters.

entry	β-aminoester	conditions[10]	conv.(%)[a]	product		substrate		E
				% yield[b]	% *ee* (*R*/*S*)[h]	% yield	% *ee* (*R*/*S*)[h]	
1	 **1**[6]	EtOAc/H_2O (98/2) 18 h	52	41	88[c] (*R*)	46	>95[f] (*S*)	>58
2		Tol/H_2O (98/2) 20 h	60	49	85[c] (*R*)	40	>95[f] (*S*)	>45
3	 **3**[6]	EtOAc/H_2O (98/2) 17 h	60	56	50[d] (*S*)	38	94[g] (*R*)	10
4	 **4**[6]	EtOAc/H_2O (98/2) 72 h	45	30	97[c] (*R*)	37	82[f] (*S*)	168
5		Tol/H_2O (98/2) 64 h	51	38	97[c] (*R*)	30	>95[f] (*S*)	>240
6	 **5**[9]	EtOAc/H_2O (98/2) 50 h	41	30	98[c] (*R*)	40	70[f] (*S*)	208
7		Tol/H_2O (98/2) 87 h	51	42	94[c] (*R*)	32	>95[f] (*S*)	>120
8	 **6**[9]	EtOAc/H_2O (98/2) 22 h	35	28	84[e] (*R*)	63	40[f] (*S*)	16
9		Tol/H_2O (98/2) 45 h	40	34	76[e] (*R*)	52	52[f] (*S*)	12

a) determined by HPLC based on disappearance of starting material; b) isolated products after flash column chromatography; c) determined by chiral HPLC using Chiralcel OD-R column and 54% CH_3CN/0.5 M $NaClO_4$in H_2O as mobile phase; d) determined by chiral HPLC using Chiralcel OG column and 7% iPrOH/hexane as mobile phase; e) determined by chiral HPLC using Chiralcel OG column and 5% iPrOH/hexane as mobile phase; f) determined by ^{1}H NMR using (*R*)-(+)-BPTPA[5] as chiral shift reagent; g) determined by ^{1}H NMR using (*R*)-(+)-1,1'-bi-2-naphthol as chiral shift reagent; h) determined according to the sign of the optical rotation and comparison with the known pure enantiomer.

However, as the enzymatic catalysis takes place in the trace water layer, the rate of reaction appeared to be strongly dependent on the hydrophobicity of the substrate. For instance, (*S*)-**5** and (*S*)-**6** could not be obtained with enantiomeric excess higher than 70% and 40% respectively (*entries 6 and 8*) because of the difficulty to drive the acylation to completion. We observed in both cases that the competing PGA-catalyzed hydrolysis of methyl phenylacetate to phenylacetic acid was faster than the catalyzed acylation. This problem was overcome by replacing EtOAc with toluene. The rate of saponification of methyl phenylacetate being considerably slower in this solvent, the acylation could be driven to >50% completion, providing (*S*)-**1**, (*S*)-**4** and (*S*)-**5** in fair yields and high enantiomeric excess (> 95%) (*entries 2, 5 and 7*). Among the five β-aminoesters studied, only **6** could not be obtained in high enantiomeric purity.

In conclusion, we have developed a practical and efficient enantioselective resolution method for racemic β-aminoesters using cross-linked ChiroCLEC™-EC (PGA) in 2% aqueous toluene. As both acylated and nonacylated enantiomers can be obtained in high enantiomeric purities and in a few steps, this method

could become a method of choice and in turn amenable for large scale preparation. We are currently investigating further applications of this methodology.

References and Notes.

1 a) "Enantioselective Synthesis of β-Aminoacids" E. Juaristi, *Wiley-VCH* **1997**; b) E. Juaristi, D. Quintana, J. Escalante, *Aldrichimica Acta* **1994**, *27*, 3; c) D. C. Cole, *Tetrahedron* **1994**, *50*, 9517-9582.

2 a) M. P. Sibi, J. J. Shay, M. Liu, C. P. Jasperse, *J. Am. Chem. Soc.* **1998**, *120*, 6615-6616; b) S. Kobayashi, H. Ishitani, M. Ueno, *J. Am. Chem. Soc.* **1998**, *120*, 431-432.

3 G. Cardillo, L. Gentilluci, A. Tolomelli, C. Tomasini, *J. Org. Chem.* **1998**, *63*, 2351-2353.

4 ChiroCLEC™-EC was purchased from Altus: 8300 U/g. PGA on Eupergit® C commercialized by Fluka was also used as catalyst and gave comparable results when used at the same concentration.

5 (*R*)-(+)-*t*-Butylphenylphosphinothioic acid: R. K. Haynes, R. N. Freeman, C. R. Mitchell, S. C. Vonwiller, *J. Org. Chem.* **1994**, *59*, 2919-2921.

6 Prepared according to: K. Eichenberger, C. Egli, Ger. Offen., 2115763 711028.

7 "Enzymes in Synthetic Organic Chemistry" C.-H. Wong, G. M. Whitesides, *Pergamon* **1994**, chapter 1.8, p. 22, and *ref.* cited therein.

8 Purchased from Aldrich.

9 The racemic compound was prepared by mixing the pure (*R*) and (*S*)-enantiomers purchased from Oxford Asymmetry.

10 A typical procedure is as follows. ChiroCLEC™-EC (111 mg of filtered water-wet material) and methyl phenylacetate (217 mg, 1.45 mmol) were added to a solution of methyl 3-amino-3-(3,4-dimethoxyphenyl)propionate (347 mg, 1.45 mmol) in EtOAc/H_2O (98/2) (15 mL). The reaction was vigorously stirred for 18 h (52% conversion based on HPLC, λ 250 nm). After filtration and recovery of the ChiroCLEC™-EC catalyst (50 mg of dry material, 286 U/mmol of substrate), the EtOAc solution was washed with 1 N HCl (1.5 mL, 1.5 mmol) diluted in water (30 mL). The EtOAc layer was washed with a saturated Na_2CO_3 solution, dried ($MgSO_4$), concentrated and purified on a short bed of silica gel (EtOAc/hexane (50/50) followed by EtOAc) to give (*R*)-methyl 3-phenylacetylamido-3-(3,4-dimethoxyphenyl)propionate **2** as a white powder (212 mg, 41%); $[\alpha]_D$ +53 (MeOH, c = 1.0); Anal. Calcd for $C_{20}H_{23}NO_5$: C 67.21, H 6.49, N 3.92; found: C 66.94, H 6.41, N 3.92. The HCl layer was basified with a saturated Na_2CO_3 solution, saturated with NaCl and extracted with EtOAc (3 x). The combined organic layers were dried ($MgSO_4$) and concentrated to give (*S*)-methyl 3-amino-3-(3,4-dimethoxyphenyl)propionate **1** as a colorless oil which crystallized on standing (160 mg, 46%); $[\alpha]_D$ +3 (MeOH, c = 1.2); Anal. Calcd for $C_{12}H_{17}NO_4$: C 60.24, H 7.16, N 5.85; found: C 59.88, H 7.38, N 5.68.