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Chemoenzymatic synthesis of enantiomerically enriched α-hydroxyamides

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Abstract

A study on a chemoenzymatic synthesis of model α-hydroxyamide was performed. Special attention was paid to the optimization of the enzymatic process, both on the selection of enzyme and cosolvent. An intriguing influence of cosolvent on the enantioselectivity of *Wheat Germ* Lipase and Amano PS Lipase catalyzed hydrolysis was observed, as the results obtained proved that enzyme's enantioselectivity is directly correlated with cosolvent's hydrophobicity. In the best example (*Wheat Germ* lipase, Et_2O used as a cosolvent), the reaction proceeded with $E = 55$, and the target compound was obtained in 33% yield with 92.7%ee.

Keywords: α-Hydroxyamides; Passerini reaction; Lipases; Enantioselectivity; Cosolvent effect

1. Introduction

Enantiopure α-hydroxyamide (A) scaffold is of great importance in organic chemistry, bearing high synthetic potential (Fig. 1). Examples of simple transformations of A lead to α-amino acids (B, $R'' = H$) and their *N*-alkylated analogues (B, $R'' = Alk$) [1], chiral α-hydroxylactams (C) [2].

Passerini multicomponent reaction has proved to be a very good method for convenient preparation of diverse α-hydroxyamides and their esters [3]. Nevertheless, Passerini reaction yields racemic mixtures, and no general solution to this problem has been established yet [4].

In our efforts to synthesize enantiopure α-hydroxyamides, we decided to use lipases for enantioselective hydrolysis of Passerini products. Lipases are widely used in biotransformation reactions, due to their wide substrate acceptance, easy accessibility and high selectivity of catalyzed transformations [5]. Therefore, they are employed in the synthesis of many various classes of compounds [6].

2. Results and discussion

Substrate for the study, ester **1**, was synthesized in Passerini reaction in 89% yield from phenylacetic aldehyde, *p*-methoxybenzyl isocyanide and acetic acid (Fig. 2). This compound was poorly soluble in phosphate buffer, what is a common problem in many enzymatic transformations.

Screening enzymatic reaction of substrate **1** was carried out with a group of enzymes. The group consisted of: *Wheat Germ* Lipase, Novozym 435A, Porcine Pancrease lipase, *Candida rugosa* Lipase, Amano AK Lipase, *Rhizopus niveus* Lipase, *Candida lypholytica* lipase, *Pseudomonas cepacia* lipase and Pig Liver esterase. Initially, acetone was used as a co-solvent, as many reports focus on water-miscible cosolvents [for example see ref. 2.a] as proper solvents for lipase-catalyzed reactions. In cited reference a series of water-miscible and immiscible cosolvents were studied and the former proved to be better in terms of enzyme's enantioselectivity. However, in our case, no product formation was observed in all screening reactions.

Therefore, we decided to focus our attention on water-immiscible cosolvents. When acetone was replaced by cyclohexane, product was obtained in the reaction catalyzed by *Wheat Germ* Lipase (Table 1, entry 3). Nevertheless, the outcome of the reaction was not satisfying, as after a long time (30

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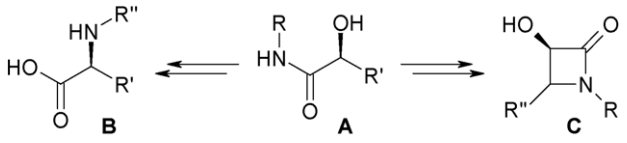

Fig. 1. Synthetic potential of α-hydroxyamides.

days) the conversion was low (26%) and enzyme's enantioselectivity ($E = 2$) was unacceptable. We therefore focused on the factors affecting the enantioselectivity of lipases. Many techniques for the increasing of enantioselectivity were established, e.g.: substrate modification [7], enantioselective inhibition of the enzyme [8] and chemical modification in the protein structure [9]. The influence of the type of water-immiscible co-solvent on the outcome of lipase-catalyzed reaction was widely reported in recent years [10–14], as it is one of the most easily altered factors in enzymatic reaction. We decided to examine the influence of the solvent in this case.

The use of less hydrophobic *iso*-propyl ether (Table 1, entry 4) gave much better results. Enantioselectivity of the reaction increased by a factor of 3. In the next step of our study, more hydrophilic solvents were used: *tert*-butyl-methyl-ether and ethyl ether (Table 1, entries 5 and 6, respectively). In the first case, a considerable increase in enantioselectivity was observed

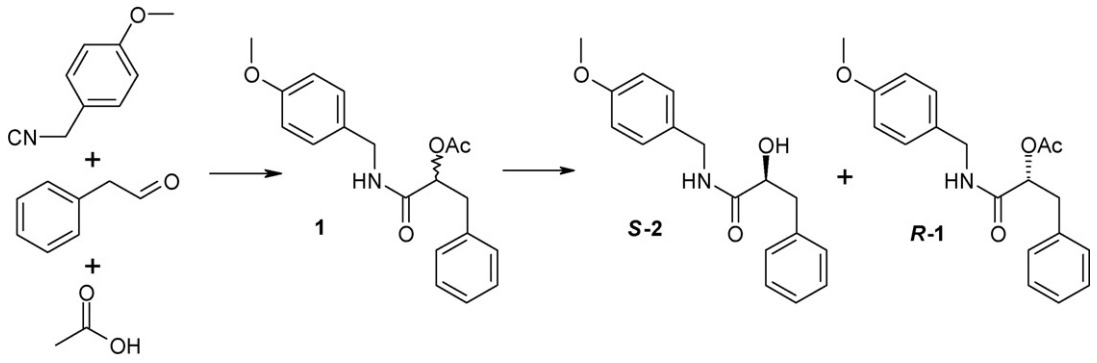

Fig. 2. Enzymatic hydrolysis of α-acetoxyamide (**1**).

Table 1
Results of enzymatic hydrolysis of **1**

	Cosolvent (log P)[a]	Time	Enzyme	Prod.	Yield (%)	ee[b] (%)	E[c] (log E)
1	–	>20 days	WGL	(*R*)-1	<1	–	–
				(*S*)-2	<1	–	
2	Me_2CO	>20 days	WGL	(*R*)-1	<1	–	–
				(*S*)-2	<1	–	
3	c-Hex (2.7)	30 days	WGL	(*R*)-1	74	3.0	2 (0.26)
				(*S*)-2	22	28.0	
4	*i*-Pr_2O (1.4)	48 h	WGL	(*R*)-1	40	58.7	6 (0.78)
				(*S*)-2	33	54.4	
5	TBME (1.0)	30 h	WGL	(*R*)-1	44	76.5	35 (1.54)
				(*S*)-2	42	87.8	
6	Et_2O (0.8)	48 h	WGL	(*R*)-1	48	69.8	55 (1.74)
				(*S*)-2	33	92.7	
7	–	>20 days	Amano PS	(*R*)-1	<1	–	–
				(*S*)-2	<1	–	
8	c-Hex (2.7)	>20 days	Amano PS	(*R*)-1	<1	–	–
				(*S*)-2	<1	–	
9	*i*-Pr_2O (1.4)	>20 days	Amano PS	(*R*)-1	<1	–	–
				(*S*)-2	<1	–	
10	TBME (1.0)	27 days	Amano PS	(*R*)-1	66	10.3	7 (0.85)
				(*S*)-2	13	73.5	
11	Et_2O (0.8)	22 days	Amano PS	(*R*)-1	57	37.1	54 (1.73)
				(*S*)-2	25	94.8	

[a] Abbreviations used: TBME, *tert*-butyl-methyl-ether; c-Hex, cyclohexane.
[b] Determined by HPLC with Daicel Chiracel OD-H column.
[c] Calculated from conversion-independent equation.

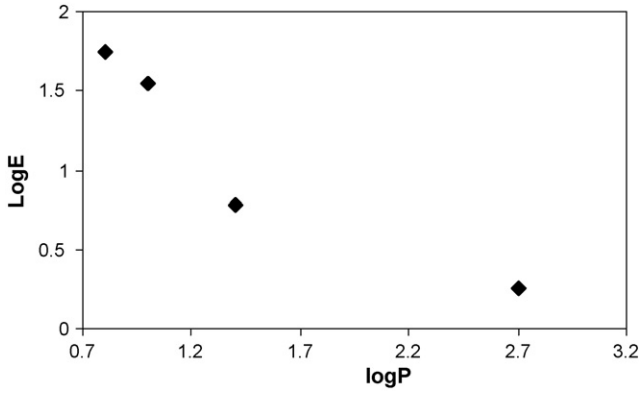

Fig. 3. Correlation between enantioselectivity of the enzymatic hydrolysis of **1** and the hydrophobicity of the solvent.

(from 2 to 35) which was even greater in the latter case (from 2 to 55). It is clearly evident that that there is a strong influence of solvent not only on enzyme activity, but also on enantioselectivity. Logarithm of enantioselectivity stays in good reciprocal correlation ($R = 0.94$) with hydrophobicity ($\log P$) of the cosolvent used (Fig. 3).

In order to verify, if the above-described correlation is also valid in case of other lipases, Amano PS lipase was also studied. Formation of product was observed when less hydrophobic solvent was used (Table 1, entry 10). As it was the case for WGL, there is a sharp increase of enantioselectivity when *tert*-butyl-methyl-ether (Table 1, entry 10) is replaced with ethyl ether (Table 1, entry 11) as a cosolvent (from 7 to 54).

Many studies were performed, and there is yet no agreement as to the mechanism [13] of the influence of cosolvent on enantioselectivity of the enzyme. Kinoshita and Ohno suggested enantioselective inhibition/activation as an interpretation [10]. Other explanations of the solvent/enantioselectivity effect include: changes in substrate conformation [11] and different interaction of solvent compounds coordinated to active site with two enantiomers of the substrate [12]. Many explanations resist on the fact that there is no correlation between co-solvent's hydrophobicity and enantioselectivity of the enzyme [11,14].

3. Conclusions

A convenient synthesis of a model α-hydroxyamide is presented. Acetic acid ester of the α-hydroxyamide was synthesized in good yield in Passerini reaction, and it was stereoselectively hydrolyzed by *Wheat Germ* Lipase (47%, 93%ee). An intriguing influence of cosolvent on the enantioselectivity of *Wheat Germ* Lipase and Amano PS Lipase catalyzed hydrolysis was observed, as the results proved a good correlation between cosolvent's hydrophobicity and enzyme's enantioselectivity.

Previous studies have shown that the influence of solvent on the enantioselectivity of lipase-catalyzed hydrolysis, although observed, was usually not this crucial (almost 30-fold increase when cyclohexane is replaced with ethyl ether). Good correlation between hydrophobicity of the solvent and enantioselectivity, and the fact that α-acetoxyamide studied by us was not entirely soluble in examined solvents, brings us to conclusion that in the studied case the shift in substrate conformation, together with strong intermolecular interactions between substrate compounds, is responsible for the solvent/enantioselectivity effect.

We believe that this phenomenon has a strong practical meaning, and will enable better optimization of lipase-catalyzed processes, what is of great importance especially in industrial processes [15].

4. Experimental

4.1. Enzyme source

Wheat Germ Lipase type I (lyophilised powder) was purchased at Sigma and Amano Lipase PS from *Pseudomonas Cepacia* was purchased at Aldrich.

4.2. Synthesis of 1

To a mixture of acetic acid (2.0 mmol, 172 μl) and phenylacetic aldehyde (2.2 mmol, 255 μl) in dichloromethane (1 ml) was added 4-methoxybenxyl isocyanide (2.14 mmol, 315 mg, dissolved in 1 ml of dichloromethane) at room temperature. After 48 h the solvent was evaporated and the product was purified by flash chromatography (Silica gel 50–100 MESH, hexanes/ethyl acetate 8:2, v/v). Yield: 89% (583 mg). mp 79–81 °C. Anal: $C_{19}H_{21}NO_4$ requires: C, 69.71%; H, 6.47%; N, 4.28%; found: C, 69.75%; H, 6.49%; N, 4.28%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05 (s, 3H, CH_3CO), 3.21 (m, 2H, CH_2NH), 3.79 (s, 3H, CH_3O), 4.33 (m, 2H, CH_2CHO), 5.41 (t, 1H, $J = 6$ Hz, CHC(O)), 6.10 (s, 1H, NH), 6.80 do 7.26 (m, 9H, 2ArH) ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.93, 37.59, 42.60, 55.23, 74.34. 113.98, 126.89, 128.37, 129.02, 129.56, 129.64, 135.74, 159.00, 168.64, 169.36.

4.3. General experimental procedure of the enzymatic hydrolysis of *1*

Substrate **1** (50 mg, 0.15 mmol) was shaken with the studied enzyme (5 mg) at 300 RPM in 10 ml of phosphate buffer 7.0 (capacity: 75 mmol/l)/organic solvent (8:2, v/v). Reaction mixture was washed with dichloromethane (3 × 8 ml). Organic phases were dried ($MgSO_4$) and the solvent was evaporated. Products were purified by flash chromatography (Silica gel 350–400 MESH, hexanes/ethyl acetate 9:1–4:6, v/v). Analysis for (*S*)-2: mp 78–80 °C. Anal: $C_{17}H_{19}NO_3$ requires: 71.56%; H, 6.71%; N, 4.91%; found: C, 71.62%; H, 6.76%; N, 4.87%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.60 (br s, 1H, O*H*), 2.90 (dd, 1H, $J = 13.9$ Hz $J = 8.2$ Hz, CH_2CHO), 3.23 (dd, 1H, $J = 13.9$ Hz $J = 4.1$ Hz, CH_2CHO), 3.72 (s, 2H, CH_3), 4.32 (m, 3H, ArCH_2N, C*H*OH), 6.77 (s, 1H, NH), 6.80 do 7.31 (m, 9H, 2ArH) ^{13}C NMR (50 MHz, $CDCl_3$): δ 41.00, 42.72, 55.43, 72.97, 114.14, 127.08, 128.82, 129.23, 129.69, 130.00, 136.90, 159.10, 172.50; enantiomeric excess (ee) was determined by HPLC equiped with Daicel Chiracel OD-H column, eluent: hexane/*iso*-propanol 9:1

(v/v), flow: 1 ml/min. Ester **1** R_t = 11.2 min (*S*), 14.2 (*R*). Alcohol 2 R_t = 8.9 min (*S*), 12.6 (*R*) [16].

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