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A Highly Selective Ester Hydrolase from *Pseudomonas Sp.* for the Enzymatic Preparation of Enantiomerically Pure Secondary Alcohols; Chiral Auxiliaries in Organic Synthesis[1]

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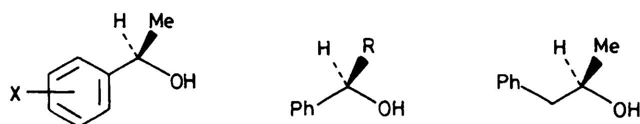
A series of valuable chiral auxiliaries, (*R*)- and (*S*)-(1)—(11), have been prepared in high chemical and optical yields by enzymatic hydrolysis of their esters in the presence of a lipase from *Pseudomonas sp.*

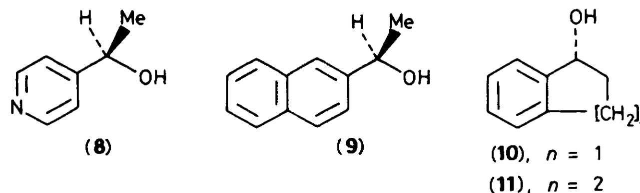
Enantiomerically pure compounds with secondary alcohol substructures are useful chiral auxiliaries in organic chemistry, both for analytical and synthetic applications.[2] Representative examples of this class such as (*R*)- and (*S*)-(1)—(11) (only one enantiomer of each compound is illustrated) can in principle be obtained by (i) classical resolution techniques *via* diastereoisomers;[3] (ii) enantioselective reductions of the corresponding ketones with chiral hydride donors,[4] micro-organisms,[5] or oxidoreductases;[6] (iii) by enantioselective hydrolysis of the corresponding esters using hydrolases or micro-organisms.[7] A critical review of these procedures reveals, however, that the hitherto available methods are often rather tedious, a fact which is reflected in the high prices of many of these compounds, several of which are not commercially available at present.

In view of the well documented, excellent ability of many ester hydrolases for enantiomer differentiation, the enzymatic hydrolysis of the corresponding esters seemed to be an obvious and facile approach to the above molecules (Scheme 1). A simple kinetic evaluation reveals that high chemical and optical yields for both enantiomers can only be obtained if their rates of hydrolysis are very different, *e.g.* by a factor of at least E = 100—500 (E is a kinetic enantiomeric ratio as defined in ref. 8). In cases of lower E values, only one enantiomer (always the remaining one) can be obtained enantiomerically pure, while the other one must be recycled.

For the last three years we have been screening for synthetically useful microbial enzymes with this desirable property[1,9] and now report highly successful experiments with an inexpensive lipase from *Pseudomonas sp.*†



(1), X = H
(2), X = *p*-Me
(3), X = *m*-Me
(4), X = *p*-MeO
(5), R = CF_3
(6), R = Et
(7)



(8) (9) (10), n = 1 (11), n = 2

† Lipase SAM-2 Amano Pharmaceutical Co., supplied by Fluka Chemie AG, CH-9470 Buchs, Switzerland (Cat. No. 62312) and Mitsubishi Int. GmbH, D-4000 Düsseldorf 1, West Germany.

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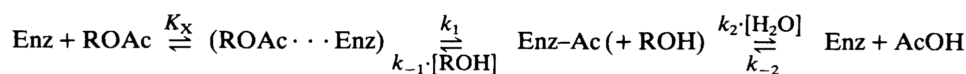
Table 1. Enzymatic hydrolysis of the racemic esters (±)-(**1**)—(**11**). *Reaction conditions*: 10 mmol substrate, 20 g pH 7 phosphate buffer (0.1 M, 20 °C), 1600 u lipase.

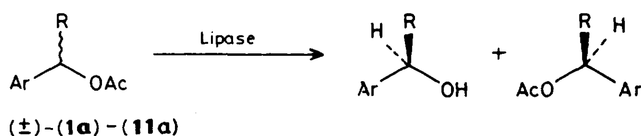
Substrate[a]	% Conversion	t/h[b]	Product	% Yield	% e.e.	Configuration	E[c]
(±)-(**1a**)	50.2	1.2	(+)-(**1**)	48	>99	*R*	>1000
			(−)-(**1a**)	48	>99	*S*	
(±)-(**2a**)	50.5	6.2	(+)-(**2**)	47	97	*R*	392
			(−)-(**2a**)	45	99	*S*	
(±)-(**3a**)	49.5	2.4	(+)-(**3**)	46	>99	*R*	>1000
			(−)-(**3a**)	46	97	*S*	
(±)-(**4a**)	50.0	9.6	(+)-(**4**)	46	80	*R*	22
			(−)-(**4a**)	47	80	*S*	
(±)-(**5b**)[d]	50.0	1.5	(+)-(**5**)	50	94	*S*	127
			(−)-(**5b**)	44	96	*R*	
(±)-(**6a**)	41.9	19.1	(+)-(**6**)	40	>99	*R*	>1000
			(−)-(**6a**)	53	72	*S*	
(±)-(**6b**)	50.0	0.5	(+)-(**6**)	46	>99	*R*	>1000
			(−)-(**6b**)	47	>99	*S*	
(±)-(**7a**)	50.0	30.0	(−)-(**7**)	47	97	*R*	270
			(+)-(**7a**)	48	97	*S*	
(±)-(**7b**)	50.0	1.0	(−)-(**7**)	45	97	*R*	270
			(+)-(**7b**)	48	97	*S*	
(±)-(**8a**)	48.4	22.5	(+)-(**8**)	46	95	*R*	117
			(−)-(**8a**)	47	89	*S*	
(±)-(**9a**)	50.0	8.4	(+)-(**9**)	45	>99	*R*	>1000
			(−)-(**9a**)	46	>99	*S*	
(±)-(**10a**)	50.0	1.3	(−)-(**10**)	46	>99	*R*	>1000
			(−)-(**10a**)	47	>99	*S*	
(±)-(**11a**)	50.0	2.4	(−)-(**11**)	47	>99	*R*	>1000
			(−)-(**11a**)	47	>99	*S*	

[a] **a** = Acetate; **b** = chloroacetate. [b] Time to 25% conversion. [c] Ref. 8. [d] Hydrolysis of (**5a**) proved too slow for any practical purpose; all experiments were therefore carried out using (**5b**).



$$\text{Enz} + \text{ROAc} \underset{}{\overset{K_X}{\rightleftharpoons}} (\text{ROAc}\cdots\text{Enz}) \underset{k_{-1}\cdot[\text{ROH}]}{\overset{k_1}{\rightleftharpoons}} \text{Enz–Ac}\,(+\,\text{ROH}) \underset{k_{-2}}{\overset{k_2\cdot[\text{H}_2\text{O}]}{\rightleftharpoons}} \text{Enz} + \text{AcOH}$$

Scheme 2



Scheme 1

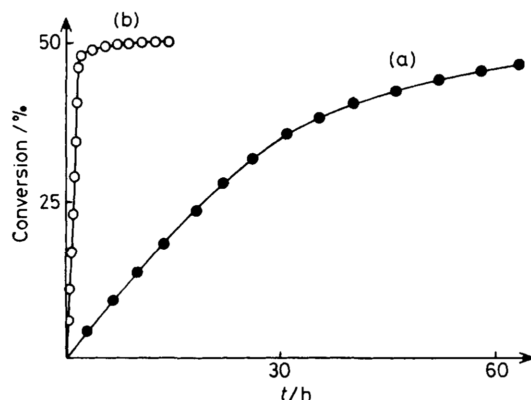


Figure 1. Hydrolysis of (a) the acetate (±)-(**6a**) (●) and (b) the chloroacetate (±)-(**6b**) (○).

In a series of experiments the racemic acetates (±)-(**1a**)—(**11a**) (10 mmol) were hydrolysed enzymatically in the usual way[9] using 0.1 M phosphate buffer (20 g, pH 7, 20 °C) and 200 mg of the lipase (1600 u; standard: tributyrin. Practically all the reactions came to near standstill after 50% conversion, as expected for a highly selective enantiomer differentiation. This is also clearly reflected in the enantiomeric purities of the obtained materials (Table 1).

Highly selective but extremely slow transformations were observed for the acetates (**5a**)—(**7a**). Conversions of 50% could not be achieved within a reasonable time, making these procedures rather impractical. A way to increase the rate of hydrolysis had to be found in order to overcome this deficiency. Reflection on the possible mechanism of enzyme action with our substrates revealed that the rate determining step could be the transformation of the enzyme–substrate complex into the acyl-enzyme (Scheme 2). Different rates of hydrolysis were observed for all the acetates, a clear indication that the hydrolysis of the acyl-enzyme is not the rate determining step. From kinetic considerations it is clear that the transformation of the enzyme–substrate complex into the acyl-enzyme (k_1) is rate determining for the overall reaction (V_{max}[8]). This assumption, which of course is only valid in the case of substrate saturation, suggests that the nucleophilic attack on the carbonyl group could indeed be rate determining. If this is so, esters activated in the acid component would show higher rates of hydrolysis.

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Indeed, we were pleased to find rate enhancements for the corresponding chloroacetates (±)-(**5b**)—(**7b**) ($ClCH_2CO_2H$, dicyclohexylcarbodiimide (DCC), 4-*N*,*N*-dimethylaminopyridine (DMAP), CH_2Cl_2, room temp.) by factors of 30—40 (see Figure 1). The *E*-values (selectivities) remained unchanged. All products were isolated by simple extraction with Et_2O followed by column chromatography on silica gel (light petroleum–Et_2O). The acetates and chloroacetates were converted into the corresponding alcohols (K_2CO_3–MeOH), the enantiomeric purities of (*R*)- and (*S*)-(**1**)—(**11**) were determined with high accuracy by g.c. separation of the isopropylurethanes on a chiral g.c. column.‡[10]

There were no indications of substrate or product inhibition in the described reactions and all these biotransformations were scaled up conveniently into the molar range.§ We feel that these results provide synthetic organic chemists with an extremely useful and facile route to a whole class of valuable chiral auxiliaries.

We are grateful to Prof. Dr. W. A. König for the determination of enantiomeric purities and the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

Received, 10th November 1987; Com. 1634

‡ XE-60-L-Valin-(*S*)-α-phenylethylamide,[10] commercially available from Chrompack Int., Middelburg, The Netherlands.

§ In a typical experiment, (±)-(**1b**) (258 g, 1.3 mol) was mixed with 0.1 M phosphate buffer (750 g, pH 7, 20 °C) and enzymatically hydrolysed in the presence of lipase (500 mg, 4000 u; standard: tributyrin). The rapidly decreasing pH, an indication of the beginning of hydrolysis, was kept constant throughout the reaction by continuous addition of 1 M NaOH solution from an autoburette. The reactions were terminated after the consumption of 650 ml base (10 h) by extraction with CH_2Cl_2. After separation of the crude product mixture by flash chromatography on SiO_2 [Et_2O–light petroleum (1:3)], (*R*)-(**1**) {76 g, 48% yield, $[\alpha]_D^{20}$ 45.0° (*c* 5.15, MeOH), 99% enantiomeric excess (e.e.)} and (*S*)-(**1b**) {121 g, 47% yield, $[\alpha]_D^{20}$ −100.2° (*c* 1.16, MeOH)} were isolated. Chemical hydrolysis (K_2CO_3–MeOH, room temp., 1 h) of (*S*)-(**1b**) yielded (*S*)-(**1**) {72 g, 97%, $[\alpha]_D^{20}$ −45.3° (*c* 5.14, MeOH), 99.5% e.e.}.

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