Enzymic Preparation of Enantiomerically Pure Secondary Alcohols. Ester Synthesis by Irreversible Acyl Transfer using a Highly Selective Ester Hydrolase from *Pseudomonas sp.*; an Attractive Alternative to Ester Hydrolysis

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A series of secondary alcohols [(*R*)- and (*S*)-(**1**)—(**3**) and (**5**)—(**9**)] of interest as attractive chiral auxiliaries were prepared in high chemical and optical yields *via* enzymic esterification under essentially anhydrous conditions by employing a highly selective ester hydrolase from *Pseudomonas sp.* and vinyl acetate as acyl donor.

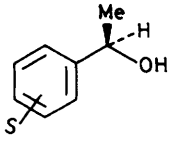
Enantiomerically pure secondary alcohols such as (*R*)- and (*S*)-(**1**)—(**9**) are useful chiral auxiliaries in organic chemistry, for both analytical and synthetic applications. The capabilities of ester hydrolases (esterases, lipases) for enantiomer differentiation are well established,[1] and the enantioselective enzymic hydrolysis or synthesis of esters derived from (±)-(**1**)—(**9**) could, in principle, provide a simple route to these molecules. Both reaction modes are reversibly catalysed by these enzymes [equation (1)]. It is further known that the intermediate acyl-enzymes [equation (2a)] can be transferred onto other nucleophiles such as the aforementioned alcohols [equation (2b)]. Since no water is involved at all, these acyl transfer reactions [in contrast to direct esterifications: equation (1)] provide essentially ideal conditions for ester synthesis. Furthermore, the application of these alternative reaction modes (hydrolysis *vs.* synthesis) has stereochemical consequences [equation (3)], producing opposite enantiomers of our target molecules. Obviously, and regardless of reaction type, high chemical and optical yields for *both* enantiomers can be achieved only if the rates of transformations differ by a factor of ≥100.[2]

$$\text{Acyl-OR} + H_2O \underset{\text{esterification}}{\overset{\text{hydrolysis}}{\rightleftharpoons}} \text{Acyl-OH} + \text{ROH} \quad (1)$$

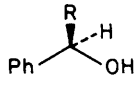
$$\text{Acyl-OR}' + \text{enzyme} \rightleftharpoons [\text{Acyl-enzyme}] + \text{R}'\text{OH} \quad \text{(a)}$$

$$[\text{Acyl-enzyme}] + \text{R}''\text{OH} \rightleftharpoons \text{Acyl-OR}'' + \text{enzyme} \quad \text{(b)} \qquad (2)$$

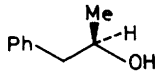
From equation (1) it is obvious that in an aqueous environment the hydrolytic reaction mode is strongly favoured; indeed we demonstrated recently,[3] employing a highly



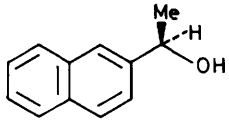
(**1**) *S* = H
(**2**) *S* = *p*-MeO
(**3**) *S* = *p*-Me



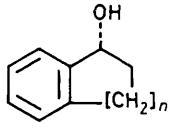
(**4**) R = CF_3
(**5**) R = Et



(**6**)



(**7**)

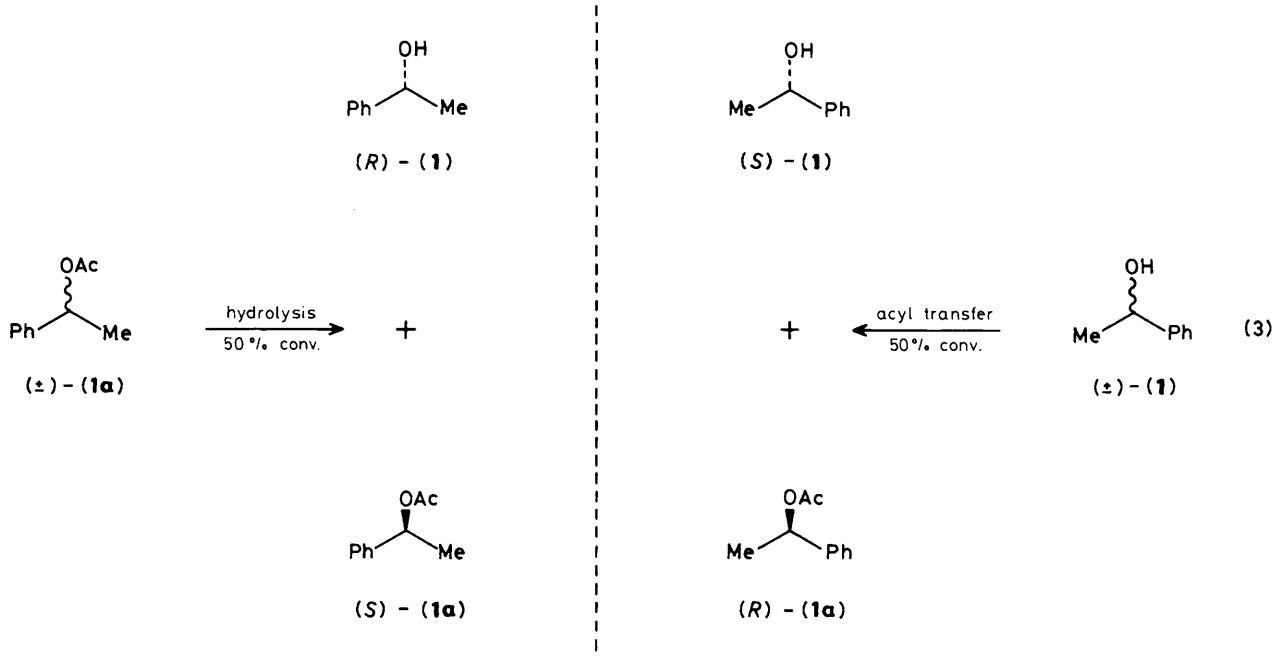


(**8**) *n* = 1
(**9**) *n* = 2

Table 1. Enzymic esterification of the alcohols (±)-(**1**)—(**9**) by irreversible acyl transfer.[a]

Substrate	Conversion (%)	Product	Yield (%)	E.e. (%)	Absolute configuration	*R*[b]
(±)-(**1**)	48	(−)-(**1**)	41	93	*S*	≥685
		(+)-(**1a**)	45	>99	*R*	
(±)-(**2**)	45	(−)-(**2**)	54	66	*S*	45
		(+)-(**2a**)	40	82	*R*	
(±)-(**3**)	48	(−)-(**3**)	47	89	*S*	≥600
		(+)-(**3a**)	40	>99	*R*	
(±)-(**4**)	No conversion					
(±)-(**5**)	26	(−)-(**5**)	68	35	*S*	≥180
		(+)-(**5a**)	24	>99	*R*	
(±)-(**6**)	30	(+)-(**6**)	66	43	*S*	≥300
		(−)-(**6a**)	31	>99	*R*	
(±)-(**7**)	43	(−)-(**7**)	53	76	*S*	≥460
		(+)-(**7a**)	41	>99	*R*	
(±)-(**8**)	49	(+)-(**8**)	48	95	*S*	≥750
		(+)-(**8a**)	46	>99	*R*	
(±)-(**9**)	50.5	(+)-(**9**)	44	>99	*S*	≥350
		(+)-(**9a**)	49	97	*R*	

[a] Reaction conditions: see text. [b] See text and ref. 2; *R* = ratio of rates of formation. The accuracy of the calculated *R*-values is strongly dependent on the exact determination of the enantiomeric purities, with the error obviously strongly increasing for $R \geq 100$.



enantioselective ester hydrolase from *Pseudomonas* sp.,† that the title compounds can be prepared with high enantiomeric purities by hydrolysis of their racemic acetates. Unfortunately, all our earlier attempts to exploit the highly attractive‡ alternative route (ester synthesis *via* acyl transfer) were unsuccessful when conventional methods were used. These involve the enzymic conversion of racemic or achiral alcohols in the presence of an ester matrix usually serving both as acyl donor and solvent solvent [*cf.* equation (2)]. Ethyl or methyl acetate, commonly used for this purpose,[4] proved totally ineffective in our case; transformations were either extremely slow or not observed at all. Clearly the alcohols (MeOH or EtOH) liberated from the ester matrix were competing effectively with our substrates for the electrophilic acyl-enzyme [equation (4)].

$$\begin{array}{l} R'OAc + \text{enzyme} \rightleftharpoons [\text{Ac-enzyme}] + R'OH \\ R' = Me, Et \qquad\qquad\qquad\qquad\qquad\qquad (4) \\ \qquad\qquad\qquad\qquad R''OH \\ R''OAc + \text{enzyme} \rightleftharpoons \end{array}$$

$$CH_2{=}CR{-}OAc + \text{enzyme} \rightleftharpoons [\text{Ac-enzyme}] + CH_3C(O)R \quad (5)$$

(**10**) R = H
(**11**) R = CH_3

Obviously, only if an irreversible route to acyl-enzymes can be used, these ester syntheses will become synthetically attractive. Enol acetates are attractive acyl donors, since the formally liberated enols are released as acetaldehyde or acetone [equations (5)].[5] Indeed we were pleased to find remarkable rate enhancements in acyl transfer reactions employing vinyl acetate (**10**) as acyl donor.§ The experimental procedures [with near stoicheiometric (!) quantities of reactants in an inert solvent (Bu^{t}OMe)] are simple, requiring only modest equipment.

In typical experiments the racemic alcohol [(±)-(**1**)—(**9**)] was dissolved in Bu^{t}OMe (15 ml) containing vinyl acetate (**10**) (5.5 mmol). After addition of the ester hydrolase (200 mg; 1600 U; standard tributyrin) the mixture was stirred at room temperature and the reaction was followed by t.l.c. or g.c. So that rates of formation could be compared, all reactions were conducted under the same conditions and terminated after exactly 44 h by removal of the enzyme (filtration), which was recovered without detectable loss of hydrolytic activity. After removal of solvent, the crude product mixtures were separated by flash chromatography on SiO_2 [Et_2O–light petroleum (1 : 3)]. Chemical hydrolysis of the esters (K_2CO_3–MeOH, room temp., 1 h) led to the corresponding alcohols, of high enantiomeric purity as determined with high accuracy by g.c. of their isopropylcarbamates on a chiral column.[6]¶ As summarized in Table 1, all product esters, with the exception of (*R*)-(**2a**) [82% enantiomer excess (e.e.)] were produced with very high enantiomeric purity (>99% e.e.). Not surprisingly, and in accord with its chemical properties the alcohol (±)-(**4**), possessing extremely low nucleophilicity, was not converted at all even under these favourable conditions; it can only be resolved enzymically by ester hydrolysis.[3]

Since, for simple kinetic reasons,[2] the observed enantiomeric purities are conversion-dependent, it was not surprising to isolate some of the remaining alcohols with considerably lower optical purities. If both enantiomers (alcohols *and* esters) are to be obtained with equally high enantiomeric purities [ideally, only one enantiomer should be esterified if the rates of formation are highly different by ≥100)] 50%

† Lipase SAM-II from Amano Pharmaceutical Co., supplied by Fluka Chemie AG, CH-9470 Buchs, Switzerland (cat. no. 62312) and Mitsubishi Int. GmbH, D-4000 Düsseldorf, Germany.

‡ Complementary to the hydrolytic reaction mode: both enantiomeric series of alcohols and acetates are accessible as desired, (±)-(**1**)—(**9**) can be used directly, no derivatisations are required, and no expensive equipment is needed.

§ In contrast to our initial expectation, the acetaldehyde produced did in no way interfere measurably with either the reaction or the enzyme; the use of isopropenyl acetate (**11**), of lower reactivity, proved less advantageous in our hands.

¶ XE-60-L-Valine (*S*)-α-phenylethylamide, commercially available from Chrompack International, Middelburg, The Netherlands.

conversions must be approached (*cf.* Table 1 for a good demonstration of this effect). Only slightly increased rates of ester formation were observed employing a larger excess (7 mmol) of (**10**); further methods for their increase are being studied in our laboratory. ‖

We feel that the present results help to increase further the flexibility and applicability of ester hydrolases. Two alternative, yet complementary and simple methods (ester hydrolysis[3] and synthesis by irreversible acyl transfer) are now available to synthetic organic chemists whereby all the enantiomers (*R*)- and (*S*)-(**1**)—(**9**) or derivatives thereof are accessible by choice and with equal ease. For additional synthetic flexibility, chemical methods for the inversion of secondary alcohols are also available.[7]

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‖ *Added in proof*: A recent Michaelis–Menten type kinetic study of the transformation of (±)-(**1**), which we believe to be the first in an aprotic medium, revealed that a considerable rate increase can be achieved by employing a 3H excess of (**10**), the reaction rate then becoming independent of the acyl donor concentration. (K. Laumen, unpublished results).

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