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ENZYMATIC ESTER HYDROLYSIS AND SYNTHESIS - TWO APPROACHES TO CYCLOALKANE DERIVATIVES OF HIGH ENANTIOMERIC PURITY

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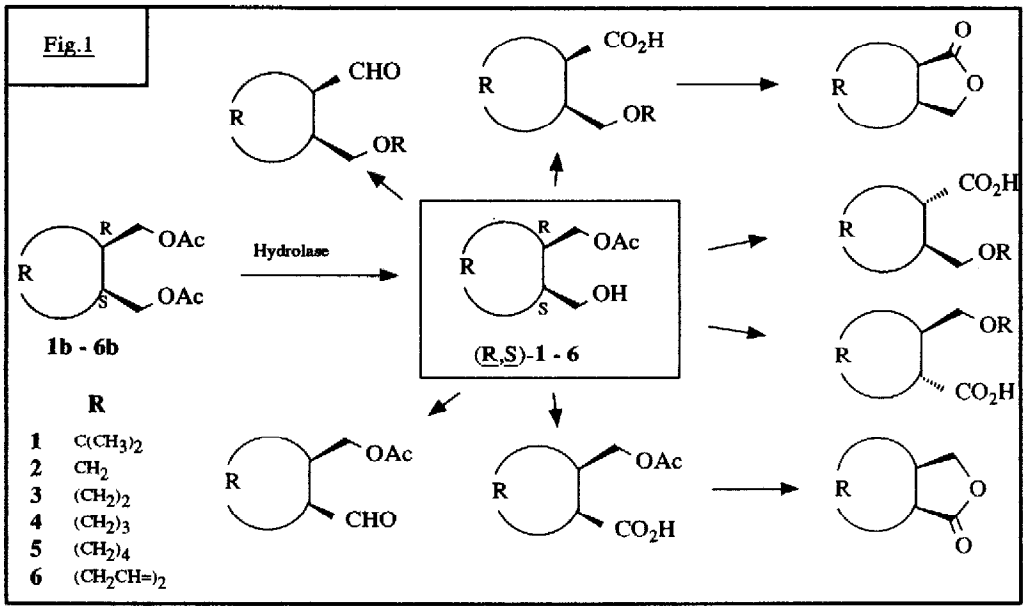
Summary : (1R,2S)- and (1S,2R)-Acetoxycycloalkanedimethanols **1-6** of high enantiomeric purities were prepared by enzymatic hydrolysis and esterification respectively in presence of lipase from porcine pancreas (PPL) and *Pseudomonas sp.* (SAM-II).

(1R,2S)- and (1S,2R)-Acetoxycycloalkanedimethanols **1-6** are attractive starting materials for a wide variety of optically active cycloalkane derivatives (Fig. 1).

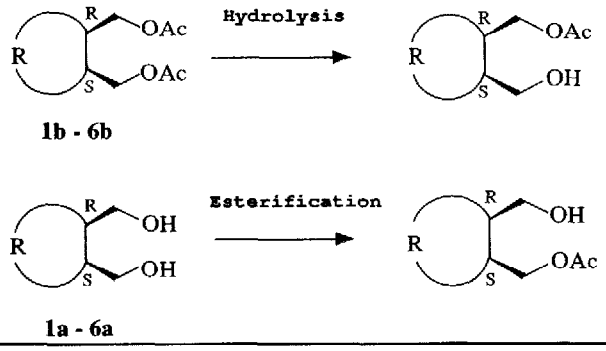


Esterhydrolases (esterases, lipases) are known a) for their capability to differentiate between enantiotopic groups in meso-substrates[1,3,6], b) to catalyze reversibly the hydrolysis of esters **and** their synthesis by direct esterification and acyl transfer (transesterification), respectively[2].

The application of these alternative reaction modes (hydrolysis vs synthesis) may have stereochemical consequences[2] and both enantiomeric series of our target molecules could therefore be accessible starting either from the corresponding diesters **1b-6b** or the diols **1a-6a** (Fig. 2).

Clearly, this strategy can only be employed successfully, leading to high chemical **and** optical yields of both enantiomers if a) the degree of differentiation between the two enantiotopic groups is high; b) the produced

Fig.2



monoacetates **1-6** are not to a large extent converted further into the corresponding diols (**1a-6a**) or diacetates (**1b-6b**), respectively.

In an aqueous environment the hydrolytic reaction mode is obviously strongly favoured and we were already partially successful earlier in hydrolyzing **1b-6b** in the presence of a crude preparation of porcine pancreatic lipase[3]. Unfortunately, however, only (1R,2S)-**6** was obtained enantiomerically pure (> 97 % e.e.) while the optical purities of the other products were less than satisfactory[4].

We are therefore pleased to report today that much better results can be achieved using a microbial ester hydrolase from *Pseudomonas sp.*[5]. In a series of experiments 20 mmol of the meso-diacetates **1b-6b** were hydrolyzed as described previously[3] employing 20 g 0.1 *M* phosphate buffer (pH 7, T=20°C) and 300 mg (9900 u, standard: tributyrin) of the enzyme. After 50 % completion (i.e. hydrolysis of one ester function), the products were isolated by continous extraction and purified by column chromatography on silica gel. The optical purities were determined by ^{1}H-NMR using Eu(tfc)$_3$ as chiral shift reagent (±3 % e.e.), absolute configurations by conversion into the known lactones (see below). The results are summarized in Table 1.

Table 1. Enzymatic hydrolysis of **1b-6b** in the presence of ester hydrolase SAM-II[a)]

Substrate	time (h)[b)]	product	yield (%)	e.e. (%)	e.e.[c)] (%)
1b	--	no conversion	--	--	(40)
2b	4.6	(1R,2S)-**2**	83	90	(74)
3b	7	(1R,2S)-**3**	87	>95	(88)
4b	9	(1R,2S)-**4**	86	>95	(86)
5b	68	(1R,2S)-**5**	54	50	(78)
6b	19.4	(1R,2S)-**6**	90	92	(95)

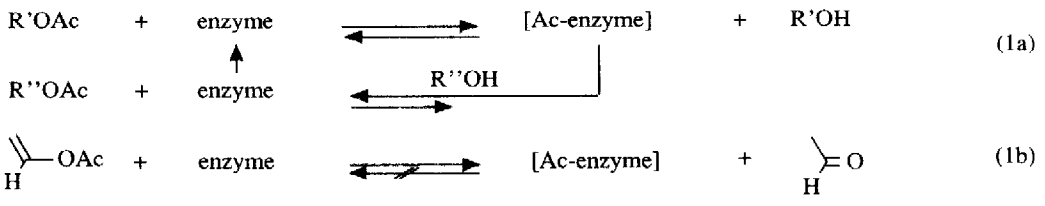
[a)] conditions see text
[b)] reaction time under standard conditions (a)
[c)] enantiomeric purities obtained previously using PPL (Ref. 3)

All products, with the exception of (1R,2S)-**1** [**1b** proved to be no substrate for SAM-II] and (1R,2S)-**5**, were obtained either with very high optical purity [(1R,2S)-**2**] or isolated essentially enantiomerically pure [(1R,2S)-**3**,**4**,**6**]. We were especially pleased to find that cis-1,2-cyclobutane- and for the first time cis-1,2-cyclopentane derivatives[1,6] are now conveniently accessible in enantiomerically pure form using ester hydrolases. (1R,2S)-5 which proved to be inaccessible directly can, of course, be obtained enantiomerically pure by hydrogenation of (1R,2S)-**6**.
Although, due to the meso-configuration of the starting materials, both enantiomeric series of derivatives can in

principle be prepared from (1R,2S)-**2-6** by selective functional group manipulation, it was attractive to see, whether an inversion of stereochemistry as outlined in Fig. 2 could be achieved by simply changing the reaction conditions from hydrolysis to esterification.

In contrast to direct esterification where, due to the production of water, unfavorable equilibria may be encountered, acyl transfer reactions (involving no water at all) are highly advantageous and provide most ideal conditions for ester synthesis. Our first attempts along these lines, using conventional methods, i.e. enzymatic conversions in an ester matrix, serving both as acyldonor and solvent[7] (e.g. MeOAc, EtOAc) proved to be only partially successful. Although, as hoped, good optical purities were obtained in some cases (Table 2, entries 2, 5, 8), only very slow transformations, requiring several days of reaction time, were observed.

Clearly the alcohols (MeOH, EtOH) liberated from the ester matrix are competing effectively with our substrates for the electrophile acyl-enzyme (eq. 1a). This problem, also encountered previously[8] was solved successfully by using an irreversible route to acyl-enzymes by employing vinylacetate as acyldonor[9] (eq. 1b).



(1a)

(1b)

In typical experiments 10 mmol of **1a-6a** were thus dissolved in 15 ml of ^{t}BuOMe containing 5.5 mmol of vinylacetate. After addition of 200 mg (6600 u, standard: tributyrin) of the enzyme (SAM-II) the mixture was stirred at room temperature, the reaction progress was monitored by g.c. Alternatively the reactions were carried out in neat vinylacetate, which served both as acyldonor and solvent. The work-up procedures are extremely facile indeed. The enzyme is simply removed by filtration and recovered without any detectable loss of hydrolytic activity. Removal of the solvent, followed by chromatography on silica gel leads to the pure products, the results being summarized in Table 2.

Table 2: Enzymatic esterification of 1a-6a by acyltransfer in presence of SAM-II[a)]

Entry	Substrate	Acyldonor	Solvent[b)]	Conversion (%)	time (h)	product	yield (%)	e.e.[c)] (%)
1	**1a**	Vinylacetate	-	--	--	--	--	--
2	**2a**	EtOAc	-	75	140	(1S,2R)-**2**	72	90
3	**2a**	Vinylacetate	-	94	6	(1S,2R)-**2**	82	>95
4	**2a**	Vinylacetate	+	95	7	(1S,2R)-**2**	80	>95
5	**3a**	EtOAc	-	86	140	(1S,2R)-**3**	82	>95
6	**3a**	Vinylacetate	-	95	5	(1S,2R)-**3**	87	88
7	**3a**	Vinylacetate	+	96	5	(1S,2R)-**3**	68	82
8	**4a**	EtOAc	-	26	48	(1S,2R)-**4**	20	>95
9	**4a**	Vinylacetate	+	95	48	(1S,2R)-**4**	85	>95
10	**5a**	EtOAc	-	12	48	--	--	--
11	**5a**	Vinylacetate	+	48	48	--	44	7[d)]
12	**6a**	EtOAc	-	14	48	(1S,2R)-**6**	--	--
13	**6a**	Vinylacetate	-	73	72	(1S,2R)-**6**	60	80[d)]
14	**6a**	Vinylacetate	+	72	96	(1S,2R)-**6**	67	88[d)]

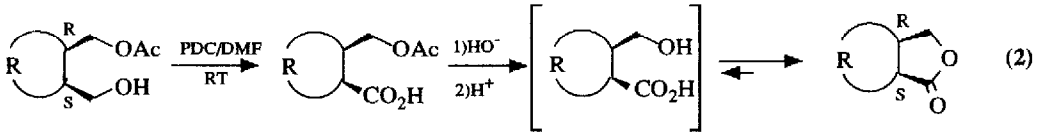
a) Conditions see text b) ^{t}BuOMe c) determined by ^{1}H-NMR using Eu(tfc)$_3$

d) large quantities of diacetates are isolated

The best results from a synthetic point of view are obtained in the esterification of **2a-4a**. Indeed and as expected (Fig. 2) the opposite enantiomers (1S,2R)-**2a-4a** are being produced, their enantiomeric are purities being very high if the appropriate reaction conditions are chosen (Table 2, entries 3,5,9). Clearly the use of vinylacetate as compared to EtOAc greatly enhances the rates of acyltransfer, some reactions being complete within only a few hours as compared to days (comp. Table 2, entries 2 and 3, 5 and 6).

Somewhat less satisfactory were the results obtained with **5a**, **6a**. While transformations in EtOAc are too slow to be of practical value, the use of vinylacetate is always accompanied with the formation of considerable quantities of the corresponding diacetates **5b**, **6b**. Next to reduced yields a decrease in enantiomeric purity is also observed in case of (1S,2R)-**6** while an almost racemic mixture is produced in the esterification of **5a**. Luckily (1S,2R)-**5** can also be prepared by catalytic hydrogenation of (1S,2R)-**6**.

The absolute configurations were unambiguously assigned by chemical correlation of (1S,2R)-**2-6** with the corresponding, known lactones **2d-5d**[10] (eq. 2).



(2)

(R,S)-**2-5** **2d-5d**

R: CH_2, $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$

In summary, both enantiomeric series of target molecules have become thus accessible by using the same enzyme under different reaction conditions. It is our feeling that this simple concept could indeed prove extremely useful in future applications and greatly enhances the scope of ester hydrolases in organic synthesis.

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