Chiral Synthons by Enantioselective Hydrolysis of *meso*-Diesters with Pig Liver Esterase: Substrate-Stereoselectivity Relationships**

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Abstract: The enantioselective synthesis of some antibiotics by combination of enzymatic and non-enzymatic procedures brought us to look at the substrate-stereoselectivity relationships in more detail. The enantiomeric excess of the chiral half-esters (Scheme 1), and even the absolute configuration of these products have been found to vary in response to small structural changes in the substrates. The present study on the substrate-stereoselectivity relationships will be useful for the rational application of pig liver esterase (PLE) in the preparation of chiral building blocks.

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**Part 20 of the Series «creation of novel chiral synthons with enzymes and applications to natural product synthesis».

During the past several years, we have shown that the enantioselective total synthesis of some antibiotics is efficiently achieved by reasonable combination of enzymatic and non-enzymatic procedures[1]. Our synthetic strategy for such optically active natural products is based on the following principle[2] (Scheme 1):

1) «Symmetrization»: retrosynthetic analysis leads from the target molecule to a *meso*-diester;
2) «Asymmetrization»: the *meso*-diester is subjected to enantioselective hydrolysis with pig liver esterase (PLE) to produce the corresponding chiral half-ester (enzymatic conversion of a σ-symmetry substrate to a C_1-symmetry intermediate, see Scheme 1);
3) «Non-enzymatic Transformation»: the chiral half-ester is converted to the target molecule or related molecules by means of «normal» organic synthesis.

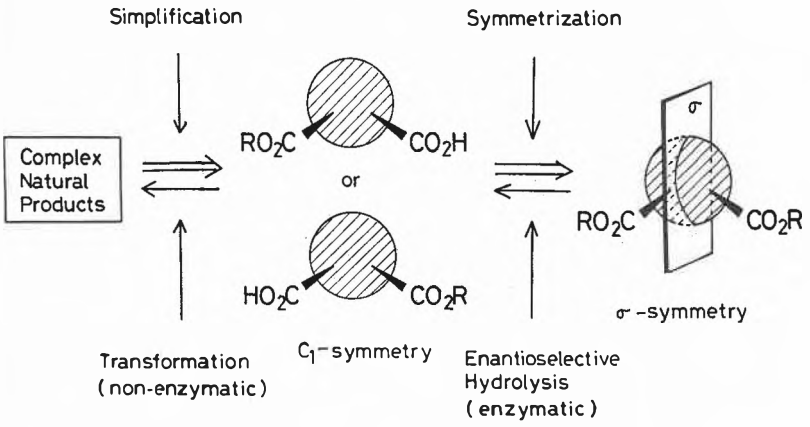
At the beginning of our enzymatic approach to natural product synthesis, we ourselves suspected that high substrate-selectivity of enzymes may be a disadvantage or difficult to overcome, to arrive at general synthetic methods for asymmetric synthesis. However, we have found that PLE, for instance, has so little substrate-specificity that it is extremely useful for this purpose. Thus, our enzymatic approach to chiral synthons has opened up a new avenue to natural product synthesis.

1. Substrate-Stereoselectivity Relationships

Asymmetric Hydrolysis of meso-Diesters

Naturally occurring β-lactam antibiotics belonging to the carbapenem group

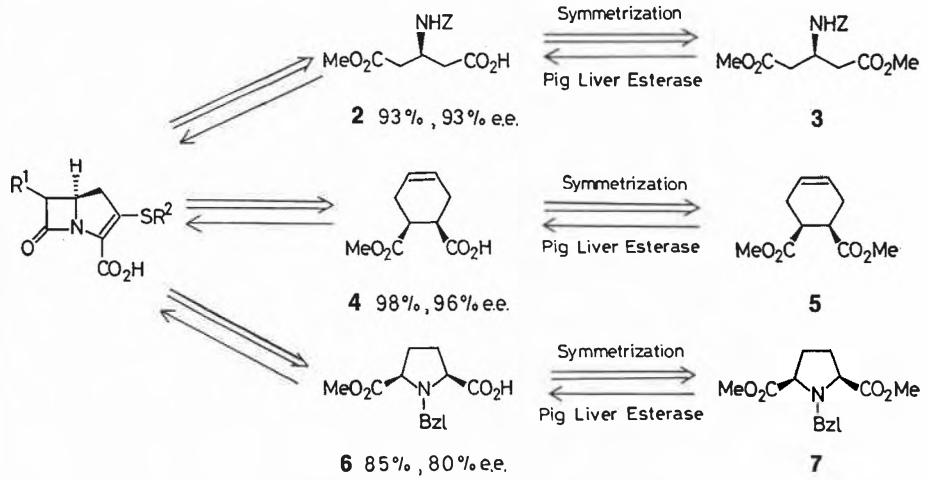
Scheme 1: «Symmetrization-Asymmetrization» Concept



have been the subject of many synthetic studies because of their unique structures and interesting biological activity. We approached the synthesis of the carbapenem «nucleus» in three different ways as shown in Scheme 2.

the half-esters is most important. What structural features influence the enantioselectivity, the absolute configuration, and the rate of the hydrolysis? The present study will give some answers to these significant questions.

Scheme 2: Diversified Synthetic Approaches to Carbapenem Antibiotics based on the «Symmetrization-Asymmetrization» Concept

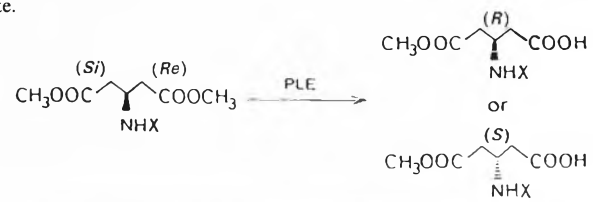


In the first approach (**3**→**2**→**1**), various types of carbapenem antibiotics were successfully synthesized[1f,g,i] and the intermediate **2** was obtained in a chemical yield of 93% with 93% enantiomeric excess (*ee*). The second approach (**5**→**4**→**1**) afforded a known intermediate to *cis*-carbapenems through the chiral half-ester **4** (yield 98%, 96% *ee*). The third approach (**7**→**6**→**1**) afforded a new chiral synthon **6** (yield 85%, 80% *ee*) although the conversion of **6** to **1** was found to be poor when compared with the other two methods. Among the three approaches to carbapenem antibiotics, the efficiency of the synthesis depends upon the availability of the *meso*-diesters, the optical purity of the chiral half-esters obtained by the enzyme-catalyzed hydrolysis, and the efficacy of the conversion to the target molecules from the half-esters. Thus the first and second route are considered more efficient. From a practical point of view of asymmetric synthesis with PLE, the optical purity of

Table 1 shows that the substituents on the amino group of the dimethyl glutarate **3** control the optical purity and even the absolute configuration of the hydrolysis-product **2**. The free aminoglutarate is easily hydrolyzed but in low optical yield (Entry 1). The optical purity of the half-esters decreases considerably with increasing chain length of the acyl groups (Entry 2 – 5), and the absolute configuration even reversed with pentanoyl groups (albeit the enantiomeric excess is very low). Bulky acyl groups afford the half-esters of *S*-configuration (Entry 6, 7, and 8). Like the saturated groups, an unsaturated small acyl group, such as acrylyl (Entry 9) affords the (*R*)-product, but with the crotonyl group the half-ester of *S*-configuration is slowly produced in almost 100% *ee* (Entry 10). Thus, crotonates should be investigated in more detail. Urethane groups afford the products of *S*-configuration, although a methoxycarbonyl group is almost of the same size as a propionyl or a acrylyl group.

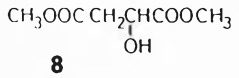
The most remarkable result is the reversal of the configuration of the preferred hydrolysis-product. The (*Re*)-methoxycarbonyl group is hydrolyzed preferentially with rather small substituents (Entry 1–4), the (*Si*)-methoxycarbonyl group with the pentanoyl group (Entry 5) and other larger substituents; such reversal was also observed with rigid cyclic substrate systems[3]. These results provide insight to the topography of the active site (binding site + catalytic site) of PLE. Synthetically, it is now possible to make a better choice of the desired *meso*-diesters by considering first the absolute configuration of the target molecule and then the expected chemical and optical yields of the chiral half-esters.

Table 1. Dependence of the absolute configuration of monoester products on the protective groups on dimethyl 3-aminoglutarate.



	X	chemical yield (%)	configuration of major half-ester	optical yield (% *ee*)
1	H	94	*R*	41
2	$COCH_3$ (Ac)	81	*R*	93
3	$COCH_2CH_3$	50	*R*	6
4	$COCH_2CH_2CH_3$	52	*R*	15
5	$COCH_2CH_2CH_2CH_3$	48	*S*	2
6	$COCH(CH_3)_2$	55	*S*	54
7	$COC(CH_3)_3$	50	*S*	93
8	COC_6H_{11}	52	*S*	79
9	$COCH=CH_2$	50	*R*	8
10	$COCH=CHCH_3$	60	*S*	≈ 100
11	COPh	59	*S*	72
12	$COOCH_3$	60	*S*	20
13	$COOCH_2CH_3$	70	*S*	40
14	$COOC(CH_3)_3$ (Boc)	93	*S*	53
15	$COOCH_2Ph$ (Z)	93	*S*	93
16	CH_2Ph (Bz)	58	*S*	33

As the next stage, we studied the regioselective hydrolysis of optically active unsymmetric diesters, because the results may give also some insight about the factors important at the active site. Thus, dimethyl malate (**8**), dimethyl aspartate (**9**), and dimethyl glutamate (**10**) were hydrolyzed with PLE. The results are shown in Table 2.



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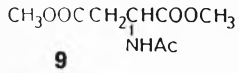

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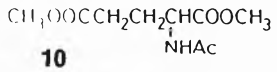

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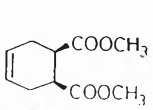
Both dimethyl L- and D-malates are readily hydrolyzed at the «A»-site although the latter one more slowly. This result may mean that the hydroxy groups of both enantiomers can bind strongly at the active site. In the case of *N*-acetyl aspartates, hydrolysis at the *β*-position is faster, and both L- and D-glutamates are hydrolyzed at the «B»-site preferentially. These results may be understood if one assumes that the less crowded methyl ester group is preferably accomodated at the active site. The results summarized in Table 2 may find some synthetic use in the regioselective hydrolysis of similar diester-derivatives.

Asymmetric Hydrolysis of Cyclic meso-Diesters

As shown in Scheme 2, the easily available dimethyl *cis*-1,2-cyclohex-4-enedicarboxylate (**5**) is efficiently hydrolyzed with PLE to afford 1-methyl-(1*S*,2*R*)-monoester[4] **4** on 98% chemical yield and 96% optical purity. The asymmetric hydrolysis can be done on 100–500 g scale very easily in a usual laboratory and the half-ester is recrystallized from a mixed solvent of *n*-hexane and diethyl ether (1:1) to afford optically pure material (recrystallization yield is about 80%). Therefore, the half-ester **4** is considered to be one of the best chiral synthons for the synthesis of natural products containing such cyclohexane systems[5]. We also investigated the dependence of the enantiomeric excess upon the nature of the ester RO group. The starting *meso*-diesters were prepared according to the method shown in Scheme 3. The results are collected in Table 3.



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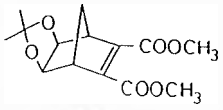

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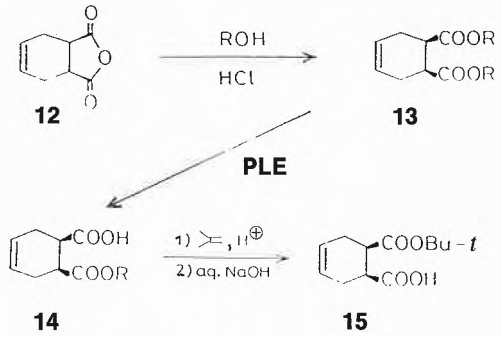
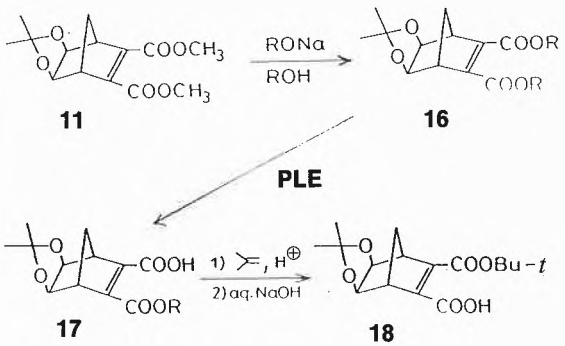
It is obvious that our success in supplying the useful chiral synthon **4** was completely dependent on the use of the methyl ester. Ethyl, *n*-propyl, and *n*-butyl esters are all very slowly hydrolyzed, in much lower chemical yields and synthetically useless optical yields. The bulky *i*-propyl ester was found to be almost completely resistent to enzymatic hydrolysis. The rather rigid cyclohexane system must be very important for binding at the active site of PLE.

On the other hand, the tricyclic *meso*-diester **11** is the starting material for the enantioselective synthesis of aristereomycin and neplanocin A[1h]. The tricyclic compound **11** was hydrolyzed to afford the half-ester with PLE in almost quantitative yield, but only with 80–88% *ee*.

Scheme 3: Preparation of meso-Diesters **13** and **16** *and Determination of the Optical Purity of the Half-esters*

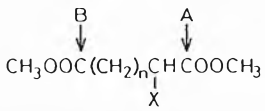


12 **13** **14** **15**



11 **16** **17** **18**

Table 2. The regioselective hydrolysis of dimethyl L- and D-malates, dimethyl L- and D-aspartates, and dimethyl L- and D-glutamates with PLE.



	n	X	*t* (h)	yield (%)	A:B
L-Mal	1	OH	1.5	66	82:18
D-Mal	1	OH	5	76	89:11
L-Asp	1	NHAc	120	39	68:32
D-Asp	1	NHAc	120	29	44:56
L-Glu	2	NHAc	120	58	7:93
D-Glu	2	NHAc	120	45	2:98

Therefore, it became necessary to improve the enantiomeric excess. We could not achieve this by using co-solvents (methanol, ethanol, dimethylformamide, tetrahydrofuran), different incubation temperatures (20–37 °C), varying pH (7.0–8.0), another protective group such as benzylidene, or no protective group at all for the glycol. Thus, the ester group was changed by treating **11** with sodium alkoxides in alcohols. The optical yield of each half-ester obtained by PLE-hydrolysis was confirmed by preparing the *tert*-butyl ester and comparing it with the authentic *tert*-butyl ester, see Scheme 3. The results are

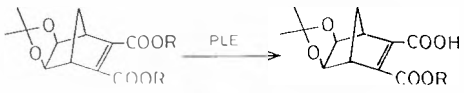
Table 3. Enantioselective hydrolysis of various *meso*-diesters **13** with PLE.

13 **14**

Substrate **13**, R =	*t* (h)	chemical yield (%)	$[\alpha]_D^{20}$ [a]	optical yield (% *ee*)
Me	3	98	+7.68°	98
Et	48	67 (26)[b]	+2.09°	27
n-Pr	48	68 (32)	+1.95°	25
i-Pr	48	5 (85)	+0.04°	2
n-Bu	48	18 (79)	+1.00°	13

[a] The optical rotations were determined with crude **15** prepared from **14** by treatment with isobutene in the presence of cat. H_2SO_4 followed by alkaline hydrolysis (see Scheme 3).
[b] Numbers in brackets show the yields of recovered material.

Table 4. Enantioselective hydrolysis of various *meso*-diesters **16** with PLE.



16 **17**

Substrate **16**, R =	*t* (h)	chemical yield (%)	$[\alpha]_D^{20}$ [a]	optical yield (% *ee*)
Me	2	100	+26.0° [a][2]	85
Et	50	30 (64)[b]	+26.4°	100
n-Pr	240	1.5 (88)	+12.0°	45
i-Pr	240	22 (58)	+10.4°	39
n-Bu	67	4.4 (88)	+19.3°	73

[a] The optical rotations were determined with crude **18** prepared from **17** by treatment with isobutene in the presence of cat. H_2SO_4 followed by alkaline hydrolysis (see Scheme 3).
[b] Numbers in brackets show the yields of recovered material.

shown in Table 4. It is obvious that the methyl ester **11** is again an exceptional substrate for PLE. The half-ester **17** (R = Me) is very easily available now, although the optical yield is 80–88% *ee*. The diethyl ester **16** is also a noteworthy substrate because the half-ester **17** (R = Et) is obtained in an almost optically pure form (≈ 100% *ee*) although the reaction is much slower (50 h). Such difficulty might be overcome by the immobilization method, if necessary. Other substrates having larger alkyl groups are shown to be impractical from a synthetic view point (much lower chemical and optical yields)[6].

2. Conclusion

We have developed a method by which a wide variety of *meso*-diester substrates undergo efficient asymmetric hydrolysis with PLE and we have demonstrated that creation of novel chiral synthons with PLE is useful for the enantioselective synthesis of natural products. It is also worthy of note that the optical yields of the chiral half-esters vary in response to even small structural variations in the substrates and the absolute configurations of some chiral half-esters may even be reversed. We hope that the rapidly increased use of PLE as catalyst in organic synthesis will give a new impact on the enantioselective synthesis of biologically significant compounds.

3. Preparation of Crude Pig Liver Esterase and the Application to the Enantioselective Hydrolysis of **16** (R = CH_3)[1h,7]

Fresh pig liver (about 4 kg) was homogenized in 18 L of cold acetone by using a kitchen juicer. After confirming that the homogenized parts became in a well-powdered state, they were collected by filtration. The residue was further washed with cold acetone (18 L) to remove the fatty material as cleanly as possible. The «acetone powder» thus obtained was dried at room temperature to afford about 1 kg of the crude pig liver. Furthermore, the fibrous materials were removed and about 800 g of fine powder was finally obtained after sieving.

Thus, 100 g of the tricyclic mesoester **16** (R = CH_3) suspended in 1 L of acetone and 10 L of 0.1 M potassium phosphate buffer (pH 8.0) was treated with 50 g of the fresh acetone powder obtained by the procedure described above at 30–35 °C for 10 h with stirring. After the reaction, the mixture was treated with hydrochloric acid to adjust the pH of the solution at 4.0. After removing insoluble materials by filtration with celite and adding sodium chloride for saturation, the filtrate was extracted with ethyl acetate several times. The organic layer was dried over sodium sulfate, and the ethyl acetate was removed. The residue was obtained in solid state. It was recrystallized from *n*-hexane/ethyl acetate to afford 75 g (79% yield) of **17** (R = CH_3); $[\alpha]_D^{25}$ −25.72°, optical purity 85%.

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[4] Cf. ref.[1l]. The absolute configuration of **4** was determined by a single crystal X-ray analysis of the derivative.
[5] The groups of *Tamm*, *Schneider*, and *Gais* also found independently that **5** is one of the best substrates for PLE. See ref.[3]; M. Schneider, N. Engel, P. Hönicke, G. Heinemann, H. Görisch, *Angew. Chem. 96* (1984) 55; *Angew. Chem. Int. Ed. Engl. 23* (1984) 67; H.J. Gais, K.L. Lukas, *ibid. 96* (1984) 140 and *23* (1984) 142.
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