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SYNTHESIS OF BOTH ENANTIOMERIC FORMS OF 2-SUBSTITUTED 1,3-PROPANEDIOL MONOACETATES STARTING FROM A COMMON PROCHIRAL PRECURSOR, USING ENZYMATIC TRANSFORMATIONS IN AQUEOUS AND IN ORGANIC MEDIA

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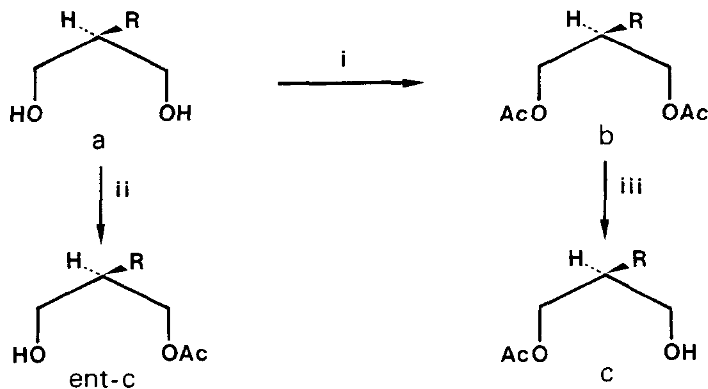
Summary: A direct entry to both enantiomeric forms c and ent-c based on enzyme catalyzed transformations of prochiral compounds of type a and b is described. The catalysts used are carboxyl esterase preparations obtained from crude porcine pancreas lipase.

The advantages of preparing chiral molecules for EPC-synthesis by way of enantioselective enzymatic transformations on prochiral starting materials are well established [1]. Our interest was focused on the enzymatic preparation of 2-substituted 1,3-propanediol monoacetates (c and ent-c) with high enantiomeric purity. Although it is possible to chemically interconvert c and ent-c, in practice this would often require the tedious manipulation of protecting groups. Commercially available porcine pancreas lipase (PPL) preparations were shown to have the same stereochemical preferences for the hydrolysis of diol mono- and diacetates [2]. These hydrolytic enzymes also catalyze acyl-transfer reactions in organic media [3]. On this basis we developed the following approach for the direct entry to both enantiomeric series c and ent-c, starting from a prochiral precursor a and using the same raw enzyme (PPL) as a catalyst source: enzymatic hydrolysis of the diacetate b produces the monoacetate c, on the other hand, enzymatic acetylation of the diol a should afford the enantiomeric monoacetate ent-c, provided that the enantioselectivity of the enzyme remains the same in organic media.



i : $(CH_3CO)_2O$ / H_2SO_4
ii : *enzymatic acylation in organic media*
iii : enzymatic hydrolysis

Herein, we present our results on the preparation of suitable catalysts and their application to compounds of type a and b with R = alkyl, alkenyl or aryl [4].

The commercially available crude PPL preparations are a mixture of enzymic and water insoluble, non enzymic materials [5].

In the case of the preparation used as a catalyst source for this work (PPL Sigma Nr. L 3126), only 6% of the solid was protein. Fast Protein Liquid Chromatography (FPLC) analysis of this material showed, besides the lipase (triacylglycerol acyl hydrolase EC 3.1.1.3 [6]) a number of other protein fractions with carboxyl esterase activity [7]. This observation, as well as the fact that the purified PPL (Sigma Nr. L 0382) was unable to hydrolyse acetate esters of type b, prompted us to fractionate the raw enzyme prior to its use for the enantioselective transformations. A sample of PPL (Sigma Nr. L 3126) was chromatographed on Sephadex G 75 and the fractions were pooled according to the activity for the hydrolysis of b to c. Analysis of the resulting four pools for the enantioselectivity of the same hydrolysis, showed that pool 1, containing the higher molecular weight fractions, was the one producing c with best optical yields [8, 9]. Comparing PPL-pool 1 with the crude enzyme, an increase of 15-40% in the e.e.-values of the obtained monoacetates was observed. For the preparative hydrolysis experiments, the preparation PPL-pool 1 was immobilized on Eupergit C [10]. The results are summarized in Table 1.

TABLE 1. Enantioselective hydrolysis of prochiral diacetates (b) catalyzed by PPL-pool 1 immobilized on Eupergit C [a].

Diacetate (b)	Monoacetate (c) (yield %)	$[\alpha]_{365}^{20}$ (c in $CHCl_3$)	e.e.% [b]
1b R : $CH_2CH_2CH{=}CH_2$	(-) 2 (80)	- 38.7° (1.00)	> 95
3b R : C_6H_5	(-) 4 (91)	- 64.3° (0.57)	> 95
5b R : $CH_2C_6H_5$	(+) 6 (65)	+ 65.3° (0.70)	61
7b R : C_6H_{11}	(-) 8 (96)	- 32.4° (0.50)	60
9b R : $CH_2C_6H_{11}$	(+/-) 10 (61)		-

a) For a typical procedure see ref. 11 .
b) e.e.-values determined by 360 MHz ^1H-NMR-spectroscopy of the corresponding (-)MTPA-derivatives.

The catalyst for transesterification was prepared by fractionation of a PPL (Sigma Nr. L 3126) solution in phosphate buffer (pH 8) with acetone, adsorbing the precipitated proteins "in situ" on Hyflo Super Cel [12]. This method affords an optimal immobilized catalyst for reactions in organic media [13]. Methylacetate, previously dried on 4Å molecular sieves, was used as the acyl donor as well as a solvent. The results are listed in Table 2.

TABLE 2. Enantioselective transesterification of diols a in methyl acetate catalyzed by a PPL fraction immobilized on Hyflo Super Cel [a].

Diol (a)	Monoacetate (ent-c) (yield %)	$[\alpha]_{365}^{20}$ (c in $CHCl_3$)	e.e.% [b]
1a R : $CH_2CH_2CH{=}CH_2$	(+) 2 (70)	+ 33.0° (0.75)	90
3a R : C_6H_5	(+) 4 (98)	+ 61.0° (0.80)	92
5a R : $CH_2C_6H_5$	(-) 6 (90)	- 13.9° (0.45)	13
7a R : C_6H_{11}	(+) 8 (90)	+ 31.0° (1.40)	58
9a R : $CH_2C_6H_{11}$	(+) 10 (90)	+ 12.8° (1.70)	10

a) For a typical procedure see ref. 14 .
b) e.e.-values determined by 360 MHz ^1H-NMR-spectroscopy of the corresponding (-)MTPA-derivatives.

Comparison of the experimental data in Tables 1 and 2 shows that the enzyme preparations used have the same enantioselectivity for the mono- and diacetate esters and that the selectivity is the same in water as in methylacetate, i.e. the acetate hydrolysis as well as the esterification take place in the same enantiotopic region of the diacetate b and the diol a, respectively [15, 16]. It is thus possible to switch the absolute configuration of the desired monoacetate by performing the reaction in the appropiate modus.

This approach, presented for 2-substituted 1,3-propanediols which are formal derivatives of 2-substituted malonic acid, is of general applicability and the described enzyme preparations can efficiently be used for the synthesis of both enantiomers of chiral, bifunctional compounds. It should be noted that chiral 2-substituted malonic acid monoesters are not available by enantioselective enzymatic hydrolysis of the corresponding diesters, due to racemization under the usual reactions conditions.

Acknowledgement: We thank Miss S. Tschumi and Mr. D. Wildermuth for their technical assistance.

NOTES AND REFERENCES

1. For a review see J. B. Jones, Tetrahedron 42, 3351 (1986).

2. Y.-F. Wong and C. J. Sih, Tetrahedron Lett. 25, 4999 (1985).

3. K. Yokozeki, S. Yamanaka, K. Takimani, K. Hirose, A. Tanaka, K. Sonomoto and S. Fukui, Eur. J. Appl. Microbiol. Biotechnol. 14, 1 (1982).

4. The diols 1a, 5a, 7a and 9a were prepared by treatment of the corresponding R-X (X : Cl or Br) derivatives with sodium diethylmalonate in abs. ethanol and subsequent reduction of the 2-substituted malonates with $LiAlH_4$ in diethyl ether. Diol 3a was prepared starting from commercially available 2-phenyl-diethylmalonate.

5. B. Borgström and H. L. Brockman, in "Lipases", Elsevier (1984).

6. Sigma porcine pancreatic triacylglycerol acyl hydrolase EC 3.1.1.3 (Sigma Nr. L 0382) of high purity (20'000-50'000 U/mg) was used as standard.

7. FPLC-analysis were performed by ion exchange chromatography on a Mono Q HR 5/5 column equilibrated with 20 mM sodium phosphate buffer (pH 8), at a flow rate of 0.5 ml/min. 200 µl samples were loaded onto the column and eluted by a 25 ml-gradient of 0 - 0.8 M NaCl in 20 mM sodium phosphate buffer (pH 8). Protein was detected by monitoring the absorbance at 280 nm.

8. Cholesterol esterase EC 3.1.1.13 (Sigma Nr. C 9530), which is present in crude pancreas preparations was also tested, showing a very poor enantioselectivity.

9. 20 g of crude porcine pancreas lipase preparation (Sigma Nr. L 3126) were dissolved in 100 ml of 69 mM sodium phosphate buffer (pH 7, buffer A). Solid material was removed by centrifugation. The clear supernatant was concentrated and then dialysed against 14 mM sodium phosphate buffer (pH 7). After removal of the precipitate by centrifugation, the sample was chromatographed on a Sephadex G 75 column (2.6 cm x 90 cm) equilibrated with buffer A. The flow rate was 10 ml/h and protein was detected by monitoring the absorbance at 280 nm. 3.3 ml fractions were collected and checked for esterase activity. Fractions 30-35, 36-75, 76-120 and 121-145 were combined in separate pools (number 1 - 4, respectively) and lyophilized.

10. A solution of 1 g PPL-pool 1 [9] in 30 ml 1 M sodium phosphate buffer (pH 7.5, buffer B) was mixed with 7 g Eupergit C (Röhm Pharma) and allowed to stay at room temperature. After 5 days the beads were filtered off, washed with 100 ml of buffer B and stored at 4°C as a buffer B suspension. The specific activity of the immobilized material was 70% of the soluble enzyme (triacetin as standard).

11. A suspension of diacetate 3b (1.86 g, 7.8 mmol) in 100 ml buffer A [9] was treated at 4°C with 200 mg PPL-pool 1 on Eupergit C [10]. After 17 h stirring at 4°C the catalyst was filtered off and stored for reuse. The filtrate was extracted with diethyl ether. The product was purified by flash chromatography (silicagel 60 Merck, 230-240 mesh, diethyl ether / pentane 2:1) and bulb to bulb distillation (90°C, 10^{-2} Torr) to give 1.41 g (91%) of monoacetate (-) 4, $[\alpha]^{20}_{365}$ = - 64.3° (c = 0.57, $CHCl_3$).

12. A centrifugated solution of 10 g crude porcine pancreas lipase preparation (Sigma Nr. L 3126) in 100 ml 18 mM sodium phosphate buffer (pH 8) was cooled at 0°C and slowly mixed with 25 g Hyflo Super Cel (Fluka Nr. 56678). To this suspension 150 ml acetone (0°C) were added dropwise within 20 min. The mixture was stirred at 0°C for additional 30 min and the Hyflo with the adsorbed protein was filtered off. The solid was dried i.V. (13 Torr) until an optimal water content of 0.1 - 1% was atteined (ca. 7 h). The catalyst was stored at 4°C. The specific activity of the immobilized preparation was 50% of the soluble enzyme (triacetin as standard). Compare also : R. A. Wisdom, P. Dunill, M. D. Lilly and A. Macrae, Enzyme Microb. Technol. 6, 443 (1984).

13. The reactions in organic solvents proceed also with raw pancreas preparation, however the reproducibility of the e.e.-values is not so good and the work-up is not so straight forward as with the immobilized enzyme.

14. A solution of diol 1a (0.5 g, 3.8 mmol) in 50 ml of methylacetate (previously dried on 4Å molecular sieves) was mixed with 2 g of PPL fraction on Hyflo Super Cel [12] and stirred for 2 h at room temperature. The catalyst was then filtered off, washed with diethyl ether (30 ml) and stored for reuse. The filtrate was evaporated and the product was purified as described in ref. 11 to yield 0.46 g (70%) of monoacetate (+) 2, $[\alpha]^{20}_{365}$ = + 33.0° (c = 0.75, $CHCl_3$).

15. In an additional experiment, hydrolysis of rac. 6 by PPL-pool 1 on Eupergit C [10] afforded (-) 6 after 50% conversion.

16. The differences in e.e.-values for the reactions in aqueous and in organic media can be attributed to the different enzyme preparations and reaction conditions. It should be noted that the enzymatic hydrolysis and the enzymatic transesterification are not the same reaction in opposite direction but two different reactions with different starting materials and "common" enantiomeric products.

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