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Enzymatic Resolution of Optically Active Aliphatic Cyanohydrins

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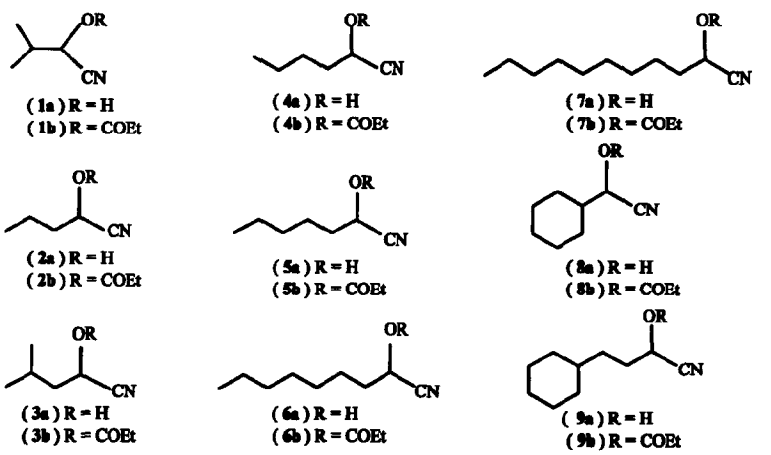
Abstract: Enantioselective acylation of cyanohydrins **1a-9a** by PPL catalysis and deacylation of propionates **1b-9b** by CCL catalysis in toluene proceed from good (E 15-20) to excellent (E > 30) enantioselectivity. A solvent has a clear effect on enzymatic enantioselectivity.

Introduction

Enantiomerically pure cyanohydrins are important starting materials for the preparation of chiral industrial chemicals, such as α-hydroxy carboxylic acids and esters or β-aminoalcohols[1]. Biocatalytic approaches to optically active cyanohydrins include the (*R*)- and (*S*)-oxynitrilase-catalysed syntheses of cyanohydrins from the corresponding aldehyde and hydrogen cyanide[1-4]. We previously described a cheap, systematic study for the synthesis of aliphatic (*R*)-cyanohydrins **1a-7a** (Scheme 1) using acetone cyanohydrin as a transcyanation agent and powdered, defatted almond meal (a rich source of (*R*)-oxynitrilase) as a catalyst[3]. The preparation of the corresponding (*S*)-cyanohydrins by (*S*)-oxynitrilase catalysis is impossible because aliphatic aldehydes are not accepted as substrates by the enzyme [4].

The lipase-catalysed resolution of racemic cyanohydrins or their acylated derivatives is an alternative biocatalytic method which has been exploited for the preparation of optically active cyanohydrins[5].



Scheme 1

In this work, we describe a systematic study for the lipase-catalysed resolution of the aliphatic cyanohydrins shown in Scheme 1. Special attention is paid for the preparation of (*S*)-cyanohydrins. Owing to the lability of cyanohydrins in aqueous solutions, the reactions have been performed in organic solvents.

Results and Discussion

In organic solvents, the preparation of optically active cyanohydrins by lipase catalysis can be performed through the deacylation of an acylated cyanohydrin or through the direct acylation of a cyanohydrin. The abilities of different lipases for the resolution of cyanohydrin **5a** with vinyl and 2,2,2-trifluoroethyl butyrates and for the resolution of propionate **5b** with hexan-1-ol were first tested in toluene (Table 1). From the lipases screened lipase PS proved to be an effective catalyst in the both types of the resolution reactions, but its enantioselectivity[6] (E = 6)) is only moderate. PPL, on the other hand, shows relatively good enantioselectivity in the acylation of aliphatic cyanohydrins. In the cases of lipase AY and CCL catalysts, enantioselectivity is good for the deacylation of propionate **5b**. This behaviour of the *Candida* lipases is exceptional in that that similar kind of enantiodiscrimination is not observed for instance for the alcoholysis of 2-octyl propionate in toluene. In accordance with our results for the CCL-catalysed acylation, only 55 % e.e.$_{(R)\text{-}2a}$ at 12 % conversion after 20 days was previously observed for the reaction between cyanohydrin **2a** and vinyl acetate in dichloromethane[5b]. The *Candida* lipases and PPL were then chosen to study enantioselective deacylations of propionates **1b-9b** and acylations of cyanohydrins **1a-9a**, respectively.

It is well documented that solvent effects on enzymatic activity may vary greatly from one enzyme to another[7]. Moreover, there is a number of examples where enzymatic enantioselectivity depends on the solvent as well[8]. Accordingly, enantioselectivity of PPL catalysis is high enough only for the acylation of

Table 1. Lipase enantioselectivities in the resolution of R*OR (0.1 mol dm^{-3})

R*	Reaction type	E			
		Lipase PS[d]	Lipase AY[d]	CCL[d]	PPL[d]
$CH_3(CH_2)_4CHCN$	Acylation[a]	6	5	5	15
	Acylation[b]	6	5	7	11
	Deacylation[c]	6	17	19	5
$CH_3(CH_2)_5CHCH_3$	Acylation[a]	9	1	1	89
	Deacylation[c]	5	1	2	5

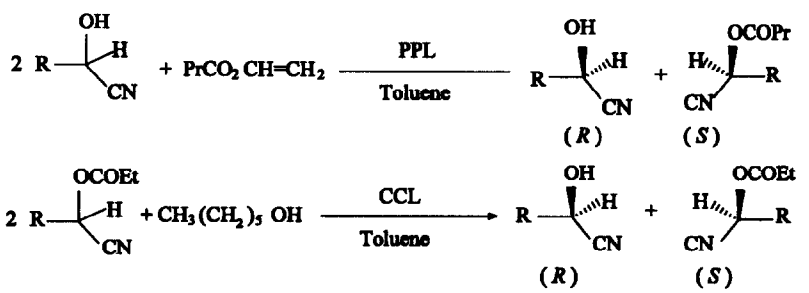
[a]Acylation with $PrCO_2CH{=}CH_2$ (0.2 mol dm^{-3}), R = H; [b]Acylation with $PrCO_2CH_2CF_3$ (0.2 mol dm^{-3}), R = H; [c]Deacylation with hexan-1-ol (0.2 mol dm^{-3}), R = OCOEt; [d]0.1 g cm^{-3}.

5a in toluene and benzene (Table 2). For the CCL-catalysed deacylation of propionate **5b** the best enantioselectivities are obtained when the reaction proceeds in diisopropyl ether, benzene or toluene (the E values 20 or higher). It can well be proposed that racemization of cyanohydrins in some of the solvents explains the solvent effects observed, but according to our chiral GLC method (Experimental Section) both optically active cyanohydrins **1a-9a** and their acylated counterparts are enantiomerically stable under the reaction conditions except in pyridine.

Table 2. Lipase enanantioselectivities in the PPL-catalysed acylation of cyanohydrin **5a** and in the CCL-catalysed deacylation of ester **5b** in organic solvents.

	Acylation[a](PPL[b])			Deacylation[c] (CCL[b])		
Solvent	Time(h)	Conversion(%)	E	Time(h)	Conversion(%)	E
Hexane	20	23	2	10	65	17
Cyclohexane	20	20	6	10	45	10
Toluene	20	30	16	10	47	20
Benzene	20	34	31	10	43	23
Dichloromethane	20	1	-	20	8	3
Diisopropyl ether	20	19	6	10	60	23
Pyridine	20	16	2	10	3	-
Tetrahydrofuran	40	21	3	10	6	2
tert-Amyl alcohol	20	7	3	10	11	2
1,4-Dioxane	40	32	4	10	6	2

[a]Acylation with $PrCO_2CH{=}CH_2$ (0.2 mol dm^{-3}); [b]0.1 g cm^{-3}; [c] Deacylation with hexan-1-ol (0.2 mol dm^{-3}).



Scheme 2

The results for the PPL-catalysed acylation of cyanohydrins **1a-9a** with vinyl butyrate in toluene are

shown in Table 3. Good (E 15-30) to excellent (E > 30) enantioselectivity of PPL catalysis is observed for cyanohydrins **2a-7a** and **9a** where the carbon chain attached to the chiral centre is not branched as is the case for compounds **1a** and **8a**. As a drawback to this method, the butyrylated (*S*)-cyanohydrins obtained are the new reaction products (Scheme 2), and for the reactions with the values of E~20 the enantiomeric excess (e.e.) for the product over 90 % will not be attained[6b]. On the other hand, the (*R*)-cyanohydrins **1a-7a** and **9a** can be prepared with higher optical purity by stopping the reactions beyond 60% conversion. This corresponds to the theoretical yield of only 40 % or less for the (*R*)-cyanohydrins compared to the yields of the order of 100 % obtained for the almond meal-catalysed condensation of HCN with the corresponding aldehyde[3].

Table 3. PPL-catalysed acylation of cyanohydrins **1a-9a** with vinyl butyrate in toluene.

Cyanohydrin	Conversion(%)	Time(h)	e.e.$^{(R)\text{-cyanohydrin}}$ (%)	e.e.$^{(S)\text{-butyrate}}$ (%)	E
1a	40	147	43	72	7
2a	56	96	92	73	20
3a	48	147	83	92	44
4a	51	96	83	81	23
5a	54	96	87	74	18
6a	55	96	88	71	17
7a	54	96	92	82	27
8a	11	162	41	5	3
9a	44	70	80	64	17

The results for the CCL- and lipase AY-catalysed hexanolyses of compounds **1b-9b** in toluene are shown in Table 4. In the case of the *Candida* enzymes, the reaction product is a cyanohydrin with the (*R*)-absolute configuration. Thus, the advantage of this method over PPL catalysis is that the (*S*)-propionates **1b-9b** now are the less reactive enantiomers and as such they can be obtained with high optical purity (e.e. close to 100 %) by using kinetic control even if the enantioselectivity is not excellent (Table 4). Enzymatic enantioselectivity in the case of the *Candida* lipases is not sensitive to the structure of the substrate. A gram-scale synthesis of propionate (*S*)-**2b** was performed using CCL catalysis in toluene. The triplicate re-use of the enzyme showed no loss in its catalytic activity nor enantioselectivity. Acylated cyanohydrins are chemically quite stable, but for the separation of free cyanohydrins with flash chromatography long treatment with silica may cause extensive racemization.

As a conclusion, CCL or lipase AY catalysts are the best methods for the preparation of aliphatic (*S*)-

cyanohydrins with high optical purity (e.e. > 90 %) using deacylation of acylated cyanohydrins in toluene, benzene or acyclic ethers, but in most other solvents the enzymatic enantioselectivity is negligible. For the CCL-catalysed alcoholysis of propionate **2b** in hexane, enzymatic enantioselectivity

Table 4. CCL- (1) and lipase AY (2)-catalysed deacylation of propionates **1b-9b** with hexan-1-ol in toluene.

Cyanohydrin (lipase)	Conversion (%)	Time (h)	e.e.$^{(R)\text{-cyanohydrin}}$ (%)	e.e.$^{(S)\text{-propionate}}$ (%)	$([\alpha]_D^{25})^a$	E
1b (1)	67	20	50	99	-77	21
1b (2)	59	7	68	97		21
2b (1)	61	20	63	99	-73	28
2b (2)	49	3	87	82		35
3b (1)	56	26	74	94	-56	16
3b (2)	60	19	65	99		26
4b (1)	58	20	72	98	-	26
4b (2)	66	20	52	99		27
5b (1)	60	22	60	97	-60[b]	15
5b (2)	62	13	62	98		19
6b (1)	62	29	61	97	-51	19
7b (1)	55	20	78	93	-40	27
7b (2)	61	20	63	98		19
8b (1)	56	22	75	96	-46	28
9b (1)	38	22	78	55	-	29

[a] $[\alpha]_D^{25}$ (*c* 3-8, benzene) for the (*S*)-enantiomer; [b] Purity of compound (*S*)-**5** 81 %.

seems slightly depend on a nucleophile, the longer octan-1-ol resulting in better enantioselectivity than butan-1-ol[5b]. PPL-catalysed acylation affords optically active acylated (*S*)-cyanohydrins with somewhat lower enantioselectivity. According to the present results and to the results obtained for the lipase-catalysed hydrolyses of acylated cyanohydrins[5], almond meal-catalysed condensation of HCN with the corresponding aldehyde is the best method for the preparation of aliphatic (*R*)-cyanohydrins[3].

Experimental

Materials. Porcine pancreatic lipase (PPL, type II, Sigma), *Candida cylindracea* lipases (CCL, type VII, Sigma and lipase AY, Amano Pharmaceuticals) and the lipase from *Pseudomonas cepacia* (Lipase

PS, Amano Pharmaceuticals) were used as received. The solvents were of the best analytical grade and were dried over molecular sieves (3 Å) before use. 2,2,2-Trifluoroethyl butyrate was prepared from butyric anhydride and 2,2,2-trifluoroethanol. Vinyl butyrate was the product of Tokyo Kasei Kogyo Co and was distilled before the use. Cyanohydrins **1a-9a** were prepared from the corresponding aldehydes and HCN using a known method[26]. The propionates **1b-9b** were produced by the reaction between the cyanohydrin and propionic anhydride in dichloromethane in the presence of pyridine and 4-dimethylaminopyridine (DMAP). The compounds were identified by ^{1}H NMR spectroscopy (400 MHz). The spectra obtained for compounds **1a-9a** are similar. The same is true with compounds **1b-9b.** As an example, the data for cyanohydrin **2a** and propionate **2b** are as follows:

2a ^{1}H NMR ($CDCl_3$): δ(ppm) 0.99 (t, 3H, CH_3, J= 8 Hz); 1.55 (m, 2H, CH_2-CH_3); 1.84 (m, 2H, CH_2-CH); 3.55 (1H, OH); 4.48 (t, 1H, CH-CN, J= 7 Hz)

2b ^{1}H NMR ($CDCl_3$): δ(ppm) 0.99 (t, 3H, CH_3-CH_2, J= 8 Hz); 1.18 (t, 3H, CH_3-CH_2CO, J= 8.5 Hz); 1.55 (m, 2H, CH_2-CH_3); 1.88 (m, 2H, CH_2-CH); 2.42 (q, 2H, CH_3-CH_2CO); 5.35 (t, 1H, CH-CN, J= 7 Hz).

The other reagents were the products of Aldrich.

Methods. The progress of the reactions and enantiomeric excesses of the prevailing enantiomers were determined by taking samples from the reaction mixture with the aid of the chiral GLC method (J&M Scientific cyclodex-β, 30 m)[9]. For that purpose free cyanohydrins in the samples were derivatized as acetates using Ac_2O and pyridine in the presence of DMAP. The base-line resolutions of the both enantiomers of cyanohydrin acetates, propionates and butyrates were excellently produced by the GLC method.

The absolute configurations of the products are based on the chiral GLC analysis and on the availability of (*R*)-cyanohydrins **1a-7a** from our previous work[3] and on the $[\alpha]_D^{25}$ -42.9 (*c* 1.02, benzene) for acetylated **1a** .

Enzymatic Reactions. The procedure for the lipase-catalysed acylation of cyanohydrins **1a-9a** and for the deacylation of propionates **1b-9b** was the same. Typically, one of the cyanohydrins or propionates (0.1 mol dm^{-3} in the reaction mixture) in an organic solvent were added on the known amount of the enzyme (0.1 g cm^{-3}). The reaction was started by adding (0.2 mol dm^{-3}) an acylating reagent in the case of acylation and a nucleophile in the case of deacylation. The reactions were performed at room temperature.

For a gram-scale resolution, a solution of racemic **2b** (0.78 g; 5 mmol) and hexan-1-ol (1.02 g ; 10 mmol) in 50 cm^{-3} of toluene were added on 3 g of CCL. The reaction was stopped at 55 % conversion after 20 h with 95 % e.e. and with $[\alpha]_D^{25}$ = -73 (*c* 3.75, benzene) for the unreacted (*S*)-**2b** and with 77 % e.e. and with $[\alpha]_D^{25}$ = +20 (*c* 5.22, benzene) for the (*R*)-cyanohydrin **2a**. 0.21 g (2.9 mmol) of propionate **2b** was first eluted followed by the elution of (*R*)-cyanohydrin **2a** using chromatography on a sintered glass funnel and diethyl ether/hexane (5/95) as an eluent.The enantiomeric excesses of compounds **2a** and **2b** were preserved during the separation.

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References and Notes

1. Kruse, C.G. in *Chirality in Industry*, Collins, A.N.; Sheldrake, G.N.; Crosby, J. Eds., John Wiley & Sons Ltd, 1992,ch. 14, p. 279.
2. a) Ziegler, T.; Hörsch, B.; Effenberger, F. *Synthesis* **1990**, 575.
   b) Smitskamp-Wilms, E.; Brussee, J; van der Gen, A. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 209.
   c) Ognyanov, V.I.; Datcheva, V.K.; Kyler, K.S. *J. Am. Chem. Soc.* **1991**, *113*, 6992.
3. Huuhtanen, T.T.; Kanerva, L.T. *Tetrahedron Asym.* **1992**, *3*, 1223.
4. a) Effenberger, F.; Hörsch, B.; Förster, S.; Ziegler, T. *Tetrahedron Lett.*. **1990**, *31*, 1249.
   b) Niedermeyer, U.; Kula, M.-R. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 386.
5 a) van Almsick, A.; Buddrus, J.; Hönicke-Schmidt, P.; Laumen, K.; Schneider, M.P. *J. Chem. Soc., Chem. Commun.* **1989**, 1391.
   b) Effenberger, F.; Gutterer, B.; Ziegler, T.; Eckhardt, E.; Aichholz, R. *Liebigs Ann. Chem.* **1991**, 47.
6. a) E = [ln(1-c)(1+ee)]/[ln(1-c)(1-ee)]; Chen, C.-S.; Sih, C.J. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695.
   b) Faber, K. in *Biotransformations in Organic Chemistry*, Springer-Verlag, **1992**, ch.2.
7. a) Klibanov, A.M. *Trends Biochem. Sci.* **1989**, *14*, 141.
   b) Kanerva, L.T.; Vihanto, J.; Halme, M.H.; Loponen, J.M.; Euranto, E.K. *Acta Chem. Scand.* **1990**, *44*, 1032.
8. Fitzpatrick, P.A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1991**, *113*, 3166.
9. N_2 carrier gas, 10 psig, detector and injector ports 280 and 250 °C, respectively. Depending on a cyanohydrin the temperature of the column was between 110 to 170 °C in an isothermal run.
10. Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *J. Org. Chem.* **1992**, *57*, 5643.