Lipase mediated kinetic and dynamic kinetic resolution of racemic 2-hydroxy-2-(5-phenylthiophene-2-yl)acetonitrile and its derivatives

Cyanohydrins are versatile building blocks in organic synthesis; their enantioselective formation has therefore attracted considerable attention.[1]The addition of HCN to carbonyl compounds is a reversible reaction and hence the starting material and the product are in equilibrium. The racemic addition of HCN to aldehydes and ketones is base catalysed. In order to suppress this racemic background reaction during the enantioselective synthesis, neutral or possibly acidic reaction conditions need to be applied.

Optically active cyanohydrins are versatile intermediates for the synthesis of a wide variety of useful compounds including amino acids,[2] hydroxy acids,[3] and others;[4] and therefore chemical[5] and enzymatic[6] synthetic methods for optically active cyanohydrins have been developed extensively. The enzymatic methods are especially attractive because high enantioselectivity can be attained easily with commercially available enzymes. The enzymatic methods reported so far can be classified into three categories: (a) the hydrocyanation of aldehydes with HCN using oxynitrilases;[6] (b) the lipase-catalyzed kinetic resolution of racemic cyanohydrins;[7] and (c) the dynamic kinetic resolution (DKR) of cyanohydrins.[7,8]

The formation of cyanohydrins from aldehydes proceeds readily. However, the equilibrium for ketones tends to lie on the side of the starting materials. The liquid acetone cyanohydrin can replace the volatile HCN, releasing it *in situ* during the synthesis of an aldehyde based cyanohydrin; thereby significantly improving safety in the laboratory.

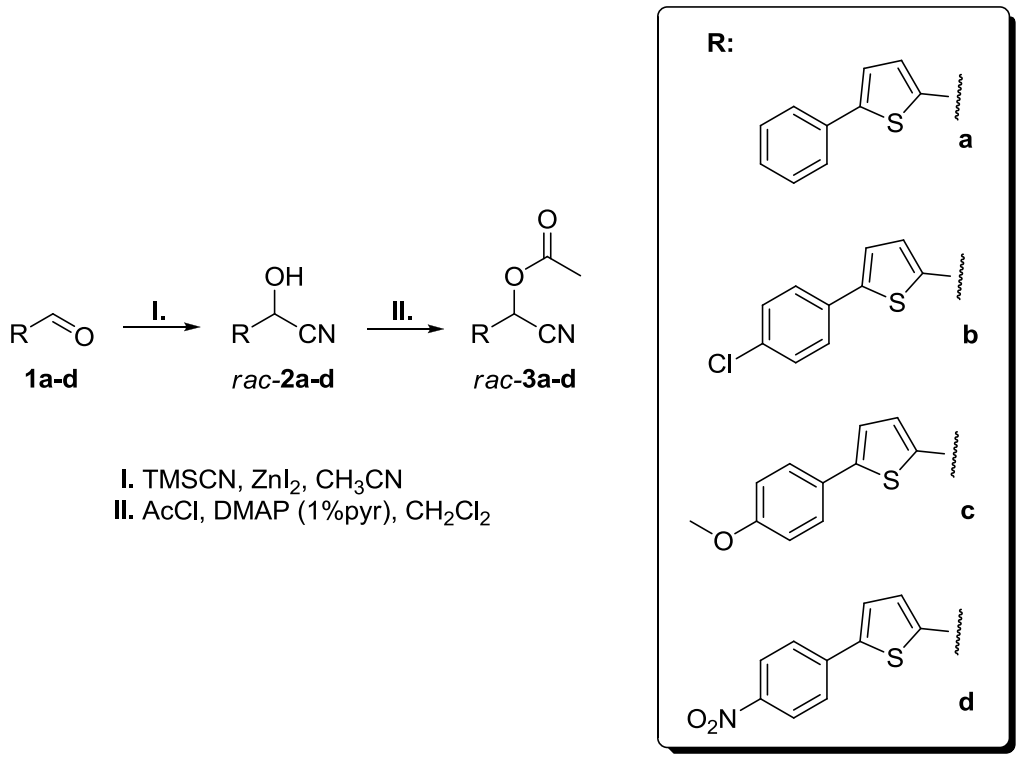
Enzymatic DKR systems have attracted much attention as an interdisciplinary technology for green sustainable chemistry.[9] Among them, the lipase-catalyzed DKR using aromatic aldehydes and acetone cyanohydrin was pioneered by Oda and co-workers in 1992.[10]

For an efficient DKR the following requirements must be fulfilled: (i) the KR must be very selective (enantiomeric ratio [E] >20); (ii) the racemization must be fast (at least 10 times faster than the enzyme catalyzed transformation of the slow reacting enantiomer); (iii) the basic catalyst must not react with the product of the reaction; and (iv) the KR and the racemization must be compatible under the same reaction conditions. Most often, the compatibility of the two catalysts is the major problem, the base might interfere with the enzyme to give poor resolution.

The base catalysed racemic synthesis of cyanohydrins is normally an undesired background reaction. However, one can also turn this dynamic equilibrium-reaction into an advantage. For instance, by combining it with the irreversible and enantioselective synthesis of a cyanohydrin ester. Acetone cyanohydrin can be used as the HCN source, since it is a tertiary alcohol, the lipases cannot convert it into an ester.[11]

2. Results and discussion

As illustrated in Scheme 1, the synthesis of the racemic cyanohydrins *rac*-**2a–d** from the corresponding aldehydes **1a–d** was performed using trimethyl silyl cyanide in the presence of a catalytic amount of anhydrous ZnI_2 in acetonitrile or dichloromethane for the synthesis of *rac*-**2d**. The racemic cyanohydrins *rac*-**2a-d** were further acylated with acetyl chloride in the presence of Py/DMAP in dichloromethane, yielding the corresponding racemic cyanohydrin acetates *rac*-**3a–d**.



Scheme 1. Chemical synthesis of cyanohydrins and their corresponding *O*-acylated esters

In order to investigate the stereoselectivity of the reactions involving chiral derivatives of 2-hydroxy-2-(5-phenylthiophen-2-yl)acetonitrile and their esters, the chromatographic separation of the enantiomers was first established (**Table 1**). The base-line separation of the enantiomers of all *rac*-**2,3a–d** was performed using a HPLC column and different mixtures of hexane-2-propanol (v/v) as eluent.

Table 1. The retention times of the enantiomers **2,3a-d**

Compound	t_r [min]	Compound	t_r [min]
(*R*)-**2a**	9.9	(*R*)-**3a**	8
(*S*)-**2a**	11	(*S*)-**3a**	9
(S)-**2b**	31	(*S*)-**3b**	19
(*R*)-**2b**	37	(*R*)-**3b**	21
(*R*)-**2c**	14.8	(*S*)-**3c**	10
(*S*)-**2c**	17.3	(*R*)-**3c**	11
(*S*)-**2d**	18.5	(*R*)-**3d**	10
(*R*)-**2d**	20	(*S*)-**3d**	11

ENZYMATIC KINETIC RESOLUTION

In order to obtain highly enantiomerically enriched (*R*)-cyano(5-heteroaryl-2+-yl)methyl acetate, commercially available immobilized lipases were screened in various organic solvents for the enantioselective acylation of the racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2a–d** with vinyl acetate as the irreversible acyl donor. First, the analytical scale enantiomer selective enzyme catalyzed acylation of racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-**2a–d** was studied using *rac*-**2a** as the model compound using vinyl acetate in the presence of different solvents and various lipases. Most of the enzymes tested such as immobilized lipase B from *Candida antarctica* (CaL-B, Novozym 435), lipases from *Pseudomonas fluorescens* (AK) on sol-gel and lipase from *Candida rugosa* (CrL) were catalytically inactive after 3 hours. The lipase from *Pseudomonas fluorescens* (AK)

immobilized by adsorbtion on Celite showed good enantioselectivity and activity (eep = 98% and ee_S=72% at c = 42% after 3 h, in MTBE). By using CaL-A from *Candida antarctica* immobilized on Celite, the reaction was faster and the selectivity was improved (ee >83%) for both reaction products at 48% conversion).
Investigating the influence of the substrate/biocatalyst ratio upon the enantioselectivity and conversion, a 1:5 (w/w) substrate/ biocatalyst ratio was found to be the most appropriate. The nature of the solvent and the nucleophile can also significantly influence the activity and selectivity of an enantioselective enzymatic acylation. Thus, the CaL-A mediated acetylation of *rac*-**2a** with vinyl acetate in several organic solvents was tested (Table 2). Acetonitrile (Table 2, entry 1) proved to be the most appropriate solvent (E = 50 at 47% conversion after 3 h). In CH2Cl2 and ethyl acetate the selectivity and the activity were moderate (Table 2, entries 2 and 3), while in MTBE and methyl THF the biocatalyst was inefficient. The CaL-A mediated acylation of *rac*-**2a** with isopropenyl acetate in acetonitrile was also studied and no significant changes for the activity and selectivity of the reaction were found compared to those obtained by using vinyl acetate (eep=81% at c=20% after 42h), also ethyl-metoxyacetate, ethyl-etoxyacetate and vinyl pivaloate were tested, but they proved to be inactive.

Table 2. Enantioselective acylation mediated by different enzymes, of *rac*-**2a** with vinyl acetate in different solvents

Entry	Enzyme	Solvent	Time (h)	c (%)	ee_S (%)	ee_P (%)	E
1	CaL-A	Acetonitrile	3	48	83	90	50
2		Ethyl acetate	3	22.7	21.5	73.1	7.9
3		MTBE	3	28.8	18.8	46.3	3.2
4		CH_2Cl_2	3	15.8	16.5	87.4	17.6
5		Methyl-THF	17	34	22.8	44.3	3.2
6	L-AK	MTBE	3	42	72	98	>200
7		Methyl-THF	3	12.6	14	98	121
8		CH_2Cl_2	17	44.6	79.8	99	>200
9	CaL-B	CH_2Cl_2	17	23	28	91	29
10	L-AKsolgel	MTBE	21	9.5	10	98	109

Table 3. Preparative scale enzymatic acylation of *rac*-**2a-d** using the best conditions found on analytical scale

Substrate	**Time (h)**	***c*(%)**	***ee*$_S$ (%)**	***ee*$_P$ (%)**	**E**
rac-**2a**	3	48	83	90	50
rac-**2b**	3	49	90	94	99
rac-**2c**	3	49	87	90	54
rac-**2d**	3	50	91	88	49

ENZYMATIC DYNAMIC KINETIC RESOLUTION

We studied the preparation of novel (*R*)-cyanohydrin acetate **3a** from the corresponding aldehyde **1a** and acetone cyanohydrin (as a source of HCN) through effective lipase-catalysed dynamic kinetic resolution of cyanohydrin **2a** with vinyl acetate (achiral acyl donor) in the presence of several bases in organic solvents (**Scheme 2B**). Vinyl acetate, one of the most

commonly used acyl donors was successfully used, although it had been previously emphasized that only isopropenyl acetate was usable.[12]
This method exploits the reversible nature of the base-catalysed cyanohydrin formation from the corresponding aldehyde **1a** and hydrogen cyanide and the base catalysed cyanohydrin decomposition to the aldehyde and hydrogen cyanide, leading to the effective racemization of cyanohydrins **2a** at the same time when the *R* enantiomer is selectively acylated in the CaL-A or L-AK- catalysed acylation (**Scheme 2**).

Table 4. Dynamic kinetic resolution of the aldehyde using the optimal conditions found for the KR and 5equiv of acetone cyanohydrin

Base	Quantity (µL)	Time (h)	ee_P (%)	ee_S (%)
triethylamine	1	6	0.5	2.2
triethylamine	0.5	4	11	2.3
triethylamine	0.5	16	1	12
diethylamine	1	15	29	70
Diethylamine (NaOH)	0.5	47	23	5
CaL-A SWCNT	10mg	6 days	99	3
Al_2O_3 90	5mg	23	99	4
Amberlite IRA-904	5mg	19	17	14
Diisopropylamine	1	13	1	3.5
Diethanolamine	3mg	3	0	2.5
Al_2O_3 basic	4mg	16	48	12

Among the bases used for the DKR with no results, there are: sepharose, diphenylamine, N,N-dimethylamine, Amberlite IR-4B, urea, guanidine, pyridine and silicagel. In order to exclude the possibility that the molecular sieve we use in our reactions for detaining the water could also promote the racemisation of our substrate and our product, respectively, we dissolved the enantiomerically enriched substrate in a solvent, we added molecular sieve and let it stirr overnight, but no racemisation occured.
We also studied the effects of temperature and additives on our DKR with triethylamine as a base catalyst and the results are presented in **Table 5**.

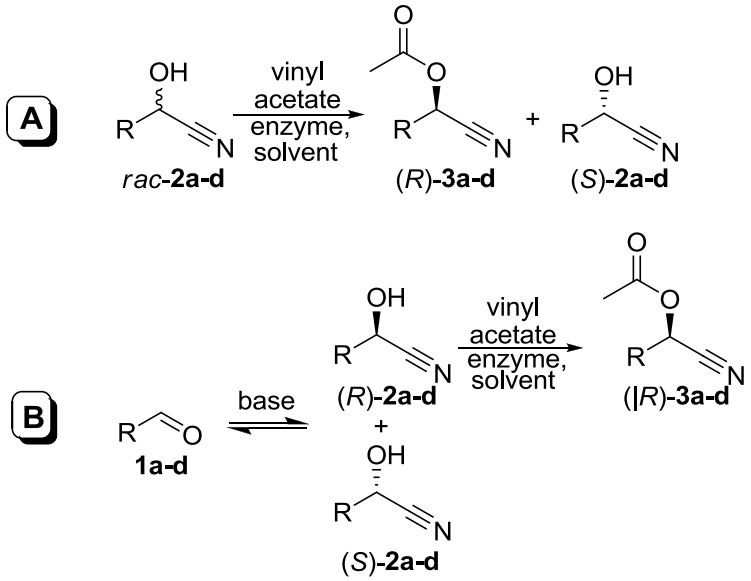
Table 5. Effect of tempretature and additives on the DKR of **2a** using triethylamine and the optimal conditions found for the KR

Additive	Temperature (°C)	Time (h)	ee_P (%)	ee_S (%)
no	40	6	1.4	0
t-butanol	25	3	35	4
acetophenone	25	3	22	1
acetophenone	25	6	8	1
K_3PO_4/ether 18C6	25	6	0.2	7
Na_2CO_3/ether 18C6	25	3	0.2	8

Possible reasons for the poor efficiency of the DKR so far, could be influenced by the amount of the base which is critical for the dynamic resolution as previously shown for the case of mandelonitrile[13]. Also, in the present work, acetic acid which is liberationg from vinyl acetate through the reaction with the water present in the enzyme preparation can neutralize the base, and accordingly lead to normal kinetic rather than dynamic kinetic resolution.

Table 5. DKR of *rac*-**2b,c,d** with vinyl acetate, L-AK in MTBE, using the racemisation agents that were found appropriate in the previous tests

Racemisation agent	Substrate	Time (h)	*c* (%)	ee_P (%)	ee_S (%)
2-Diethylaminoethanol acetylated	*rac*-**2b**	3	43.5	95.6	73.6
		17	17.5	37.7	8
	rac-**2c**		no product		
	rac-**2d**	3	60	63.6	95.8
		17	65.6	50.6	96.9
Al_2O_3 basic	*rac*-**2b**	17	3.8	79.3	3.2
	rac-**2c**		no product		
	rac-**2d**	17	46.2	74.7	64.3
Triethylamine	*rac*-**2b**	3	48.7	87.9	83.4
		17	47	40	36
	rac-**2c**	3	46.4	99	86.6
		17	99	0	72
	rac-**2d**		racemic product		



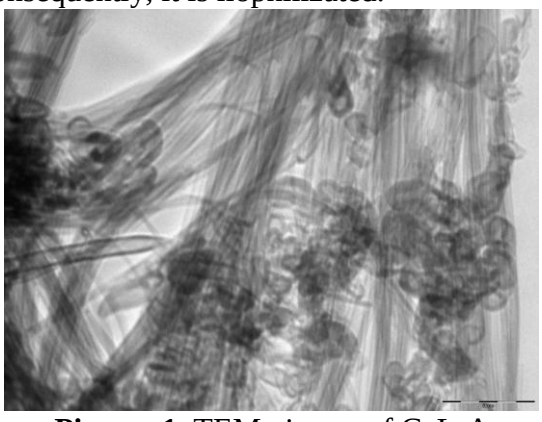
Scheme 2. A- enzymatic acetylation of the racemic cyanohydrins *rac*-**2a-d**
B- one pot synthesis of (*R*)-**3a-d** by dynamic resolution

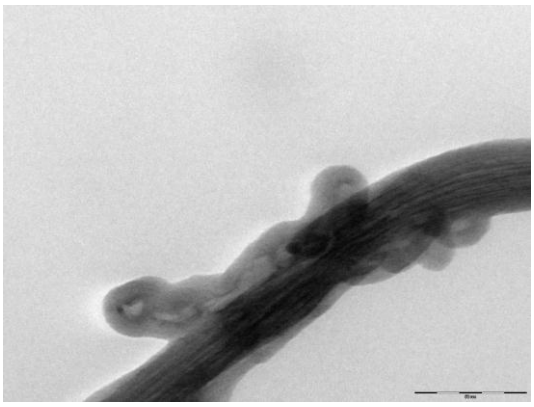
Experimental

High Performance Liquid Chromatography (HPLC) analyses were conducted with an Agilent 1200 instrument (Table 1). The enantiomeric separation of *rac*-**2,3a** and *rac*-**2c** was performed on a Chiralpak IA column (4.6 × 250 mm) and a mixture of n-hexane and 2-propanol 90:10 (v/v) as eluent; *rac*-**2,3b** was separated on a Chiralpak IC column and a mixture of n-hexane and 2-propanol 97:3 (v/v) as eluent, *rac*-**2d** was separated on a Chiralpak AS-H column and a mixture of n-hexane and 2-propanol 50:50 (v/v) as eluent (**2d** adsorbed best at 320nm), *rac*-**3d** was separated on a WELK column and a mixture of n-hexane and 2-propanol 90-10 (v/v) as eluent and *rac*-**3c** was separated on a Chiralpak IB column and a mixture of n-hexane and 2-propanol 90-10 (v/v) as eluent. Thin layer chromatography (TLC) was carried out using Merck Kieselgel $60F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63 - 200

μm). The determination of E was based on equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$, with $c = ee_S/(ee_S + ee_P)$.
All reagents were purchased from Aldrich or Fluka and used as received. Solvents and acyl donors for enzymatic reactions were stored over molecular sieves unless otherwise stated. Lipases from *Pseudomonas fluorescens* (AK) immobilized by adsorbtion on Celite or sol-gel, and lipase from *Candida rugosa* (CrL), were purchased from Amano, England. Immobilized lipase B from *Candida antarctica* (CaL-B, Novozyme 435) was purchased from Novozyme, Denmark. Lipase A from *Candida antarctica* immobilized by adsorbtion on Celite (CaL-A) was a gift from Professor Liisa T. Kanerva, University of Turku, Finland.

1. Synthesis of racemic 2-heteroaryl-2-hydroxyacetonitrile *rac*-2a–d
Using a previously described method[14], a catalytic amount of anhydrous ZnI_2 (3.2 mg, 20 μmol) and trimethylsilyl cyanide (160μL, 1.2 mmol) was added to a stirred solution of one of the aldehydes **1a–d** (1 mmol) in dry acetonitrile or dichloromethane (5 mL) and the resulting mixture was stirred at room temperature until all of the aldehyde was transformed. The solvent was evaporated and the crude product was redissolved in 5 mL of methanol. The formed trimethylsilyl cyanohydrin decomposed when HCl (3 M, 3 mL) was added. The reaction mass was evaporated to the final volume of 3 mL, after which water (5 mL) and ethyl acetate (10 mL) were added. After the separation of the two layers, the aqueous layer was extracted with ethyl acetate (2 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. The crude mixture was purified by recrystallization from CH_2Cl_2 or by column chromatography using CH_2Cl_2: Acetic acid 99:1 as eluent.
2. Synthesis of racemic cyano(5-heteroaryl-2-yl)methyl acetate *rac*-3a-d
To a solution of one of the racemic cyanohydrins *rac*-**2a–d** (1 mmol) in dry dichloromethane (5 mL), acetyl chloride (4 mmol, 284 μL) and a catalytic amount of DMAP in pyridine (150 μL, 1% solution) were added. After stirring overnight at room temperature, the solvent was evaporated in vacuo and the crude product was purified by column chromatography using dichloromethane as eluent.
3. Enzyme mediated biotransformations
A. Enzyme immobilization on carbon nanotubes
L-AK, CaL-A and CaL-B were separately immobilized on single wall carbon nanotubes (SWCNT) by adding 50mg of SWCNT, 3mL of phosphate buffer (50mM) pH 7.6 and 100mg of enzyme dissolved in 1mL of the same buffer; the obtained mixture is sonicated for 30 minutes, then stirred at rt and 1350rpm for three hours and then it is frozen at -20ºC and consequently, it is liophilizated.


Picture 1. TEM picture of CaL-A immobilized on SWCNT


Picture 2. TEM picture of L-AK enzyme immobilized on SWCNT

B. Enzymatic kinetic resolution of rac-2a–d with vinyl acetate and different lipases
To a solution of *rac*-**2a–d** (2.5 mg) in an organic solvent, vinyl acetate (4 μL) and the lipase (12.5 mg) were added. The reaction mixture was shaken, over molecular sieves, for 3 h at room temperature. For HPLC analysis, samples taken from the reaction mixture (40 μL) were diluted to 600 μL with 2-propanol and filtered before injection.
C. Dynamic kinetic resolution of 1a-d
Aldehydes **1a-d** (0.013 mmol, 2.5mg), acetone cyanohydrin (0.065 mmol, 5μL), a base and vinyl acetate (0.052 mmol, 4.9 μL) were mixed with CaL-A on Celite in acetonitrile to allow DKR at 25 ^{o}C and 1340rpm. For HPLC analysis, samples taken from the reaction mixture (40 μL) were diluted to 600 μL with 2-propanol and filtered before injection.

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