Optimisation of the Enantioselective Synthesis of Cyanohydrin Esters

Lars Veum,[a] Liisa T. Kanerva,[b] Peter J. Halling,[c] Thomas Maschmeyer,[d] Ulf Hanefeld[a,*]

[a] Gebouw voor Scheikunde, Technische Universiteit Delft, Julianalaan 136, 2628 BL Delft, The Netherlands
Fax: (+31)-15-278-4289, e-mail: u.hanefeld@tnw.tudelft.nl
[b] Laboratory of Synthetic Drug Chemistry, University of Turku, Lemminkäisenkatu 5 C, FIN-20520 Turku, Finland
Fax: (+358)-2-333-7955, e-mail: lkanerva@utu.fi
[c] Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, United Kingdom
Fax: (+44)-141-548-4822, e-mail: p.j.halling@strath.ac.uk
[d] Laboratory of Advanced Catalysis for Sustainability, School of Chemistry, Building F11, The University of Sydney, NSW, 2006, Australia
Fax: (+61)-2-9351-3329, e-mail: th.maschmeyer@chem.usyd.edu.au

Received: January 21, 2005; Accepted: March 25, 2005

Abstract: The base- and lipase-catalysed enantioselective synthesis of cyanohydrin esters was investigated, and the problem of previously reported low yields due to residual water in the reaction mixture was addressed. When the lipase was immobilised on Celite R-633 as a carrier, both the enantioselectivity and the reaction times for this dynamic kinetic resolution were improved, thus enabling a highly enantioselective synthesis of aromatic and heteroaromatic cyanohydrin acetates.

Keywords: asymmetric synthesis; Celite; dynamic kinetic resolution; enzyme catalysis; lipase B from *Candida antarctica*

Introduction

Chiral cyanohydrins can be converted into a wide range of compounds, which are versatile building blocks for the synthesis of fine chemicals, pharmaceuticals and agrochemicals, and they are therefore an important subject of research.[1–3] There are four main approaches for the synthesis of enantiopure cyanohydrins: either chemically, using cyclic dipeptides or transition metal complexes, or enzymatically, using hydroxynitrile lyases (HNLs) or lipases.[1]

The enzyme-catalysed methods are of great interest due to their low costs and environmentally friendly aspects. In the HNL-catalysed additions of HCN to aldehydes and ketones, both excellent yields and enantiomeric purities were achieved within short reaction times (<1 h).[4–9] However, the reaction is mostly performed in a two-phase system, so water-sensitive and water-insoluble substrates can only be used with difficulties. Since the reaction is an equilibrium reaction, a large excess of HCN or of the less toxic acetone cyanohydrin is needed to obtain high conversions. Furthermore, not all substrates are accepted by the HNLs and some of the resulting cyanohydrins racemise very easily. As an alternative to the HNLs, one can use lipases. They have been applied both in the kinetic and in the dynamic kinetic resolution (DKR) of cyanohydrins (Scheme 1).[1,10] While the kinetic resolution has a maximum yield of 50% only, and involves at least two separate steps to reach higher yields, the DKR starting from a prochiral aldehyde yields theoretically 100% in just one step.[11]

The DKR combines a base-catalysed equilibrium between an aldehyde, acetone cyanohydrin and the resulting cyanohydrin of the aldehyde, and a lipase-catalysed acylation of one enantiomer of this cyanohydrin. As the remaining enantiomer of the cyanohydrin is racemised by the base, one can theoretically obtain a 100% yield of the acylated cyanohydrin. This reaction was already published in 1991,[12–14] but, in spite of its elegance, it has only been used successfully in a limited number of cases.[15–19] This might be due to the fact that hydroxynitrile lyase-catalysed cyanohydrin synthesis tends to be faster and give higher yields and enantiomeric purities for most of the substrates that have been studied.[4] But this could also be caused by the unsuccessful examples of the DKR that have been reported.[20–22]

It has previously been shown that one of the most widely applied lipases in synthesis, *Candida antarctica* lipase B immobilised on a methacrylate polymer (Novozyme 435, or Chirazyme L-2, *cf.* C2, Lyo; both abbreviations stand for the same enzyme preparation), is par-

 DOI: 10.1002/adsc.200505031

ticularly enantioselective in the kinetic resolution of cyanohydrins.[22–24] In order to increase the utility of the DKR, we recently investigated the reaction. Starting from **1a**, using Novozyme 435, basic Amberlite was used as the racemisation catalyst.[22] The racemisation of mandelonitrile (**2a**), the transcyanation between the acetone cyanohydrin and the aldehyde, as well as the kinetic resolution of mandelonitrile *via* acylation, all worked well separately. However, when the three reactions were combined, the reaction hardly even went to 16% conversion in the DKR. It was concluded that the water present in the system hydrolysed the acylating agent, yielding an acid, which in turn neutralised the base that catalyses the dynamic equilibrium. Eventually, the acid might even render the enzyme inactive. HCN formed during the reaction could have an equivalent effect. If simply more base was added, another problem arose, namely the base-catalysed polymerisation of HCN. These polymers inhibited the enzyme and led to a full stop in the reaction.

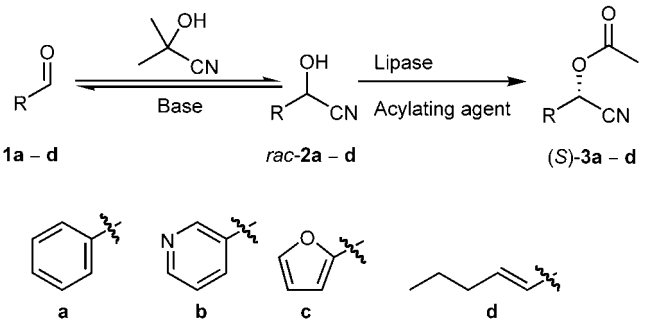
Results and Discussion

In this work we now look at five different possibilities to solve this problem. Solid buffers have previously proven to be useful in establishing and maintaining optimum acid-base conditions for enzymes in organic media.[25] We use the shorthand "pH" to refer to this, although in the absence of a liquid water phase, simple pH is not the appropriate parameter. If solid buffers are used in the DKR, they should help in ensuring a favourable "pH", buffering any acetic acid formed. Another option is to use cyanide salts as bases. They should also neutralise any acetic acid formed in the reaction, yielding HCN that in turn should add to the aldehyde. In the first description of the DKR, molecular sieves were used to dry the reaction.[12–14] We look at this possibility, too, even though molecular sieves also work as ion exchangers, and therefore may alter the "pH" of the reaction in an unfavourable direction.[26] The carrier of the enzyme may also be of importance. As Celite has been used as the carrier in all the successful applications of the DKR, it is also of interest to compare Novozyme 435 with CAL-B immobilised on Celite. In addition it is known that Celite R-640 can be used to control water activities in organic media.[27] It can therefore be expected that the Celite used for the immobilisation has this effect on the reaction, too. We chose Celite R-633 in this study since we have applied it successfully in the immobilisation of *Hb*HNL before.[28] The acylating agent is known to have an effect on both the enantioselectivity and the activity of the enzyme and the possibility of using other acylating agents was explored.[10]

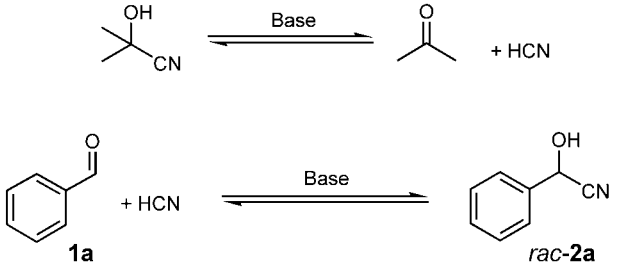
Based on the first DKR reported,[12–14] 1 mmol of aldehyde, 2 mmol of acetone cyanohydrin, and 3 mmol of acetylating agent were used in combination with various amounts of base and Novozyme 435 in 4 mL of toluene at 40 ^{0}C. In our previous work we could show that 380 U of Novozyme 435 were sufficient for the kinetic resolution of **2a** to proceed to 50% conversion within 4 h and with an excellent E (>100). Therefore 380 U/mmol substrate were employed for the reaction[22] (Scheme 1).

Solid buffers have previously been used to adjust the ionisation state of enzymes in organic media, and to maintain it.[25] It was therefore investigated whether these solid buffers could replace the traditionally used Amberlite (Amberlite IRA-904 in OH^- form). Since fast racemisation of the non-acylated enantiomer of **2** is essential for the DKR (to reach 100% conversion with a high enantioselectivity), we tested various solid buffers (MOPS, HEPES, HEPPSO, AMPSO and CAPS) as catalysts for the dynamic equilibrium (Scheme 2, Table 1). Only CAPS gave results that were similar to Amberlite, but CAPSO too catalysed this reaction within a reasonable time frame. Based on these results, CAPS, CAPSO and Amberlite were used for our further studies. The active catalyst in the buffer pair is probably the solid Na salt, which has a free basic amino group.

In order to find the optimal quantity of base to use in the DKR starting from **1a** and **1b**, various amounts of CAPSO, CAPS (40, 60, 80, 100, 120 mg of each salt of the buffer pair), Amberlite (20, 40, 100, 200, 300 mg, 1 mmol OH^-/g Amberlite) and 380 U of Novozyme



Scheme 1. Enantioselective synthesis of cyanohydrin esters *via* a dynamic kinetic resolution.



Scheme 2. Base-catalysed equilibrium between acetone cyanohydrin, HCN and acetone and between **1a**, HCN and **2a**.

Table 1. pK_a and time to reach the base-catalysed equilibrium between **1a**, acetone cyanohydrin, **2a** and acetone.

Solid buffer	pK_a	T_{eq}[a]
MOPS	7.2	no reaction
HEPES	7.5	>30 h
HEPPSO	7.8	no reaction
TAPS	8.4	24 h
AMPSO	9.0	30 h
CAPSO	9.6	5 h
CAPS	10.4	10 min
Amberlite		20 min

[a] Time to reach equilibrium with 100 mg of buffer.

435 per mmol substrate were used. The reactions were analysed after 7 days.

In the case of CAPSO, an optimal conversion is reached for both **1a** (conversion 100%, ee 91%) and **1b** (conversion 66%, ee 74%) when 100 mg of each salt of the pair are used. With CAPS the maximum conversions were already achieved when only 60 mg of each salt of the pair were used (**1a:** conversion 98%, ee 91% and **1b:** conversion 57%, ee 26%). However, for **1b** the conversions and ees were slightly lower than with CAPSO.

Amberlite is a strong base and, in the case of **1a** and **1b**, the conversion drops slightly when more base is added. This is probably due to polymerisation of HCN and a consequential deactivation of the enzyme.

For **1a**, the ee is generally only slightly reduced if more base is used in the reactions. But in the case where the amount of Amberlite OH^- utilised in combination with **1b** increases from 40 mg to 200 mg, the ee for **3b** decreases from 70% to 20%. This might be due to the fact that **1b** is also a base in itself, aiding the chemical background reaction or the base-catalysed racemisation of the product.

All the experiments with the solid buffers were repeated using molecular sieves to dry the reaction mixture. However, independent of how much buffer or Amberlite was used, the molecular sieves did not influence the reaction significantly: neither the conversion nor the ee obtained showed much deviation from the results obtained without molecular sieves.

In the original paper describing the DKR, both Amberlite conditioned with NaOH and NaCN were used. Even though the two gave comparable results, the OH^- form was chosen because it gave a slightly higher ee. Instead of using CN^--conditioned Amberlite, we explored the possibility of employing different cyanide salts. The idea is that any acetic acid formed as a side product in the reaction will be neutralised by the cyanide salt. The HCN formed will then add to the aldehyde while the metal salt of the acetate precipitates. In order to pair the soft cyanide anion with a hard, intermediate and soft acid, we chose for the salts NaCN, $Zn(CN)_2$ and CuCN, respectively, and used these as bases/salts in parallel with Amberlite OH^- and NaOAc in the DKR starting from **1a** and **1b** (Table 2). The composition of reagents remained unchanged; i.e., 2 equivalents of acetone cyanohydrin were added.

As expected there is a correlation between the hardness of the acid and the enantioselectivity. According to the hard-soft acid-base principle, the bond in CuCN has a covalent character, NaCN is completely ionic while $Zn(CN)_2$ is an intermediate between the two. Consequently, the CN^- is more "available" as a base in NaCN than in CuCN. As predicted this trend is evident in the results. NaCN catalyses the reaction, but in the case of the more reactive substrates the ee is low due to the base-catalysed acylation of the cyanohydrin or the base-catalysed racemisation of the product. CuCN is a poor catalyst, and it only works in the case of **1b**. However, as **1b** is a base in itself, the observed conversion might also be autocatalysed. $Zn(CN)_2$ gives by far the best ees but only a moderate conversion. Both NaOAc and Amberlite gave higher conversions than $Zn(CN)_2$, but, the ees were lower.

These experiments were repeated in the presence of molecular sieves as drying agents. As expected there was no significant difference between the two sets of experiments, whether molecular sieves are used or not. Any acid is neutralised by the cyanide salt to form HCN and the corresponding salt of the acid. As long as there is cyanide salt present, the "pH" of the reaction should be constant and the molecular sieves should not act as ion exchangers.

In all the successful, albeit sometimes slow, DKR of cyanohydrins, the lipases were immobilised on Celite. Celites are natural silicates, and some of them can adsorb large amounts of water. They bind this water tightly *via* hydrogen bridges; indeed Celite R-640 can be used to

Table 2. The use of salts as bases in the DKR starting from 0.98 mmol **1a** and **1b**, using 380 U/mmol substrate of Novozyme 435, after 6 days.

Compound	NaCN[a, b]	$Zn(CN)_2$[a, b]	CuCN[a, b]	NaOAc[a.b]	Amberlite[a, c]
1a	90 (36)	24 (94)	0 (–)	41 (89)	55 (90)
1b	96 (0)	25 (85)	3 (91)	32 (35)	63 (57)

[a] Conversion (ee) [%].
[b] 1 equivalent.
[c] 0.3 equivalents.

efficiently control low water activities in organic solvents, since it can adsorb more than 90% of the Celite's weight.[27] In comparison, Novozyme 435 is adsorbed on a divinylbenzene-cross-linked, hydrophobic macroporous polymer based on methyl and butyl methacrylic esters.[29] This lipophilic material will readily release any water that is attached to it into the dry reaction mixture, thereby enabling the hydrolysis of both the product and the acyl donor. Not all types of Celite are suitable for enzyme immobilisations and care has been taken to choose a suitable one. We immobilised CAL-B on Celite Biocatalyst carrier R-633 according to standard procedures[30] and tested it with substrates **1a**, **1c**, **1d** and the bases NaCN, $Zn(CN)_2$, NaOAc and Amberlite. The results obtained after 6 days are shown in Table 3.

The observed trend is the same as when Novozyme 435 is used. However, if one considers both conversion and enantiomeric excess, Amberlite is clearly the best base to use. The result we obtained for **1c** is a significant improvement over what has earlier been reported. In the original work on the DKR, a conversion of 73% of **1c** to its corresponding cyanohydrin acetate **3c** was achieved with an ee of 47% in 6 days using Amberlite as the base. The results that are reported here for **1a** are also an improvement compared to those obtained earlier with Novozyme 435, both in terms of conversion and enantiopurity.

In order to see how CAL-B on Celite R-633 (910 KU/mmol substrate) performed in combination with the solid buffers, it was used for the synthesis of **3a**, together with various amounts of base (CAPSO, CAPS) (Table 4).

Although CAPSO gave fairly high ees, a substantial amount of buffer is necessary to obtain the same conversion as with CAPS. The conversion that is obtained with CAPS is not influenced by how much base is used, however, the ee drops as a function of the amount of base added. These differences can be rationalised by the base strength of the solid buffers. More CAPSO is needed for the racemisation since it is the weaker base, while larger amounts of the stronger base CAPS lead to product racemisation. When performing the same reaction using 30 mg of Amberlite, 83% conversion and an ee of 97% were obtained. This is better than what was obtained with both CAPSO and CAPS. These results cannot be directly compared with those obtained with Novozyme 435 since less units of the enzyme were employed in those experiments.

However, it is clear that Amberlite in combination with CAL-B on Celite R-633 not only gives good yields but also an excellent enantioselectivity. In order to directly compare the two different lipase immobilisations, we tested various amounts of both the CAL-B on Celite R-633 and Novozyme 435 in the synthesis of **3a** (Figure 1).

The results clearly demonstrate that even if the same amount of activity is used, the CAL-B adsorbed on Celite R-633 performs significantly better, both in respect to yield and enantioselectivity. In the case of CAL-B on Celite R-633 the conversion increases with an increasing amount of enzyme, until a plateau is reached, indicating that the enzymatic reaction is no longer the rate-limiting step. The slight drop in ee for **3a** is consistent with this, indicating that the dynamic equilibrium now is the rate-limiting step.[31] It also shows that the 380 U, which were enough for the kinetic resolution of **2a** to proceed rapidly, are not enough for the DKR to proceed smoothly: at least 1.3 KU/mmol substrate have to be used. To ensure that it is really the effect of the carrier that causes the difference described in Figure 1, and not the immo-

Table 3. The use of salts as bases in the DKR starting from **1a**, **1c** and **1d** using CAL-B on Celite R-633 (380 U), after 6 days.

Compound	NaCN[a, b]	$Zn(CN)_2$[a, b]	Amberlite[a, c]	NaOAc[a, b]
1a	–	18(92)	87(95)	56(91)
1c	69(4)	23(75)	97(86)	71(84)
1d	76(11)	10(81)	71(79)	28(74)

[a] Conversion (ee) [%].
[b] 1 equivalent.
[c] 0.3 equivalents.

Table 4. The use of various amounts of CAPSO and CAPS as bases in the DKR starting from **1a** using CAL-B on Celite R-633 (910 U/mmol substrate) with a reaction time of 48 hours.

Mass acid/base [mg]	CAPSO[a]	CAPS[a]	Amberlite[a, b]
-/30	–	–	83 (97)
40/40	48 (95)	87 (91)	–
60/60	52 (95)	85 (82)	–
80/80	78 (91)	85 (79)	–
100/100	86 (92)	86 (77)	–

[a] Conversion (ee) [%].
[b] 0.3 equivalents.

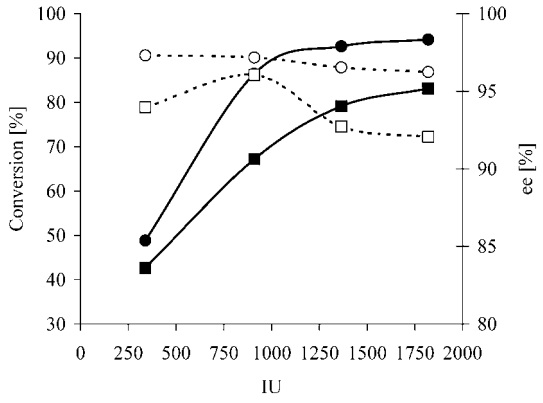


Figure 1. The yield of **3a** after 3 days using Amberlite OH^- as the base, isopropenyl acetate as the acylating agent, and various amounts of Novozyme 435 (conversion ■, ee □) were tested vs. CAL-B on Celite R-633 (conversion ●, ee ○).

bilisation procedure, we stirred the Novozyme 435 in the same buffer-sucrose solution that was used for the immobilisation of CAL-B on Celite R-633 and dried it in the same way. The treated Novozyme 435 showed no difference compared to the untreated Novozyme 435 preparation.

Even though vinyl acetate and vinyl butanoate give acetaldehyde as a side product, which can in general be harmful for enzymes,[10] and can act as a substrate for the DKR, they have still been the acylating agents of choice in several of the reported successful applications of the DKR. We tested isopropenyl acetate, vinyl acetate and vinyl butanoate with CAL-B adsorbed on Celite R-633, and, after 16 hours the conversions and ees were 35 (98), 13 (83) and 14 (92) respectively. Since CAL-B in combination with isopropenyl acetate gives both the best conversion and enantiomeric excess, all further studies were performed with this acylating agent.

The reaction was performed with 1350 U/mmol CAL-B on Celite R-633 on a preparative scale, starting from **1a** and **1c**; using Amberlite as the base and isopropenyl acetate as the acylating agent (Table 5).

Table 5. Results of the DKR starting from **1a** and **1c** using optimised reaction conditions.

Substrate	Time [days]	Yield [%]	ee [%]
1a	4.0	97	98
1c	4.5	92	89

For both **1a** and **1c** the preparative reaction proceeded within four days, which is faster than described earlier (4 and 4.5 days rather than 6 days for both **3a** and **3c**, respectively). More importantly, the enantioselectivity for both compounds was significantly improved. For **1c** the yield is increased from 57% to 92%, and the enantiomeric excess is increased from 47% to 89%. For **1a**, it is now an almost enantiospecific reaction (ee = 98%), instead of an enantioselective reaction (ee = 84%).[13] When comparing it to the vanadium-salen-catalysed formation of **3a** from **1a**, KCN and acetic anhydride it also is a significant step forward. Although the vanadium-catalysed reaction proceeds faster (10 h), the yield (88%) and the enantioselectivity (ee = 90%) are lower.[32]

Work on the DKR starting from aliphatic aldehydes is in progress. Preliminary results indicate that the system that is described in this paper is not the best solution. The optimisation for these substrates will be reported elsewhere.

Conclusion

This work describes the synthesis of cyanohydrins *via* a DKR using the readily available lipase B from *Candida antarctica*. The problems experienced earlier, of low conversions and long reaction times due to hydrolysis of the acylating agents, have been addressed and solved.

Varying the nature and the amount of base used in the DKR of cyanohydrins already gave improvements compared to previous work using Novozyme 435, although a straightforward change in the carrier of the enzyme had the largest effect on the reaction. Whereas Novozyme 435 probably releases water from the carrier into the reaction media, causing hydrolysis which subsequently stopped the reaction, Celite R-633 suppressed the negative side effects of water most likely by binding it. With lipase B from *Candida antarctica* adsorbed on Celite R-633, both the conversion and the ee was improved significantly when compared to earlier results.

Experimental Section

General Remarks

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 300 (300 MHz and 75, MHz, respectively), instrument. Chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Optical rotations were obtained using a PerkinElmer 241 polarimeter. Column chromatography was carried out with silica gel 0.060–0.200 mm, pore diameter *ca.* 6 nm. TLC was performed on 0.20 mm silica gel and developed in a vanillin bath [vanillin (15 g) in ethanol (250 ml) + concentrated H_2SO_4 (2.5 mL)] where all products containing a CN group gave orange spots. Dry toluene was purchased from Aldrich. Lyophilised lipase B from *Candida antarctica* was purchased from Roche. Immobilised lipase B from *Candida antarctica* (CALB, Novozyme) was a generous gift from Novo Nordisk (Dr. Deussen). Lyophilised CAL-B was adsorbed on Celite Bio-Catalyst Carrier R-633 from World Minerals.[30] In the original procedure Celite® (Filter agent) from Aldrich was used. The activity of the enzymes used was determined as described earlier.[24] Amberlite IRA-904 was conditioned with NaOH,[13] and the basicity of the conditioned ion exchanger was found to be 1 mmol OH^-/g. All aldehydes, acetone cyanohydrin and isopropenyl acetate were distilled prior to use and stored under nitrogen. The racemic cyanohydrin acetates **3a**–**f** were prepared according to a standard procedure[33] and their spectroscopic data correspond to those in the literature.[14,24,34–36] All reactions were performed in a 10-mL glass vial equipped with a silicone supported teflon septum and a screw cap. 1,3,5-Triisopropylbenzene was used as an internal standard in the reactions.

HPLC Analysis

The HPLC analysis were performed using a 4.6 × 250 mm Chiracel OB-H column with a Waters 510 pump, and a Waters 486 UV detector. The eluent was a 90:10 mixture of hexane and 2-propanol containing 0.1% acetic acid. The flow was 0.8 mL min^{-1}.

Table 6. Temperature programs and retention times for **1a–d**, (*R*)-**3a–d** and (*S*)-**3a–d**.

Substrate	Temp. [°C][a]	R_t [min] **1**	R_t [min] (*R*)-**3**	R_t [min] (*S*)-**3**
a	145	1.00	2.62	3.12
b	145	0.99	3.89	5.05
c	120	1.00	3.04	3.83
d	120	0.99	2.96	3.91

[a] All analyses were performed isothermally.

GC Analysis

The conversion and enantiomeric purity were determined by chiral GC using a β-cyclodextrin column (CP-Chirasil-Dex CB 25 m × 0.25 mm) using a Shimadzu Gas Chromatograph GC-17A equipped with a FID detector and a Shimadzu Auto-injector AOC-20i, using He with a linear gas velocity of 75 cm/s as the carrier gas. The temperature programs and retention times are given in Table 6.

Base-Catalysed Transcyanation from Acetone Cyanohydrin to Benzaldehyde

Acetone cyanohydrin (0.17 ml, 1.84 mmol) was added to a stirred mixture of benzaldehyde (98 mg, 0.92 mmol) and either the solid buffer (50 mg of both the acid and its corresponding sodium salt) or Amberlite (30 mg), in toluene (4 mL) at 40 °C. Samples (10 μL) were diluted in hexane and analysed by HPLC.

General Procedure A: The DKR of Cyanohydrins, Analytical Scale

Acetone cyanohydrin (0.17 mL, 1.84 mmol) was added to a stirred mixture of the aldehyde (0.98 mmol), the acylating agent (2.76 mmol), the immobilised enzyme (as stated in the text) and the base (as stated in the text) in dry toluene (4 mL). The reaction was stirred magnetically at 40 °C. Samples (10 μL) were diluted in acetone and centrifuged. The supernatant was analysed by chiral GC.

General Procedure B: The DKR of Cyanohydrins, Preparative Scale

Acetone cyanohydrin (0.86 mL, 9.4 mmol) was added to a stirred mixture of the aldehyde (4.7 mmol), isopropenyl acetate (1.62 ml, 14.9 mmol), CAL-B on Celite R-633 (6.83 KU) and Amberlite IRA-904 in OH^- form (150 mg) in dry toluene (20 mL). When the reaction was completed, the enzyme and the resin was filtered off and washed with toluene (2 × 10 mL). The solvents were removed under vacuum and the residue was purified by column chromatography on silica gel (PE/EtOAc, 90:10).

(*S*)-(−)-2-Acetoxy-2-phenylethanenitrile [(*S*)-3a]

The title compound was prepared from benzaldehyde (499 mg, 4.7 mmol), following general procedure B. (*S*)-**3a** was isolated as a clear oil; yield: 798 mg (97%); 98% ee; $[\alpha]_D^{20}$: −6.2 (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3 CO), 6.40 (s, 1H, CH-O), 7.20–7.58 (m, 5H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.5 (CH_3CO), 62.9 (CH-O), 116.2 (CN), 127.9 (C-2,6), 129.3 (C-3,5), 130.4 (C4), 131.8 (C-1), 169.9 (C=O).

(*S*)-(−)-2-Acetoxy-2-(2-furyl)ethanenitrile [(S)-3c]

The title compound was prepared from furfural (452 mg, 4.7 mmol), following general procedure B. (*S*)-**3d** was isolated as a clear oil; yield: 718 mg (92%); 89%; $[\alpha]_D^{25}$: +22.4 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300MmHz, $CDCl_3$): δ = 2.14 (s, 3H, CH_3 C=O), 6.47 (m, 2H, CH-CN and C3-H), 6.69 (m, 1H, C2-H), 7.51 (m, C4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.2 (CH_3), 55.8 (CH-O), 111.2 and 112.7 (C-2 or C-3), 114.3 (CN), 144.2 (C-1), 145.1 (C-4), 168.8 (C=O).

Acknowledgements

U. H. thanks the Royal Netherlands Academy of Arts and Sciences (KNAW) for a Fellowship. The authors gratefully acknowledge COST D25 for enabling their collaboration.

References and Notes

[1] M. North, *Tetrahedron: Asymmetry* **2003**, *14*, 147–176.
[2] H. Griengl, H. Schwab, M. Fechter, *Trends Biotechnol.* **2000**, *18*, 252–256.
[3] J. Brussee, A. van der Gen, in: *Stereoselective Biocatalysis*, (Ed.: P. N. Ramesh), Marcel Dekker, Inc., New York, **2000**, pp. 289–320.
[4] R. J. H. Gregory, *Chem. Rev.* **1999**, *99*, 3649–3682.
[5] H. Griengl, A. Hickel, D. V. Johnson, C. Kratky, M. Schmidt, H. Schwab, *Chem. Commun*. **1997**, 1933–1949.
[6] F. Effenberger, *Chimia* **1999**, *53*, 3–10.
[7] M. Schmidt, H. Griengl, *Top. Curr. Chem*. **1999**, *200*, 193–226.
[8] G. Seoane, *Curr. Org. Chem*. **2000**, *4*, 283–304.
[9] F. Effenberger, S. Förster, H. Wajant, *Curr. Opin. Biotechnol*. **2000**, *11*, 532–539.
[10] U. Hanefeld, *Org. Biomol. Chem.* **2003**, *1*, 2405–2415.
[11] J. Sukumaran, U. Hanefeld, *Chem. Soc. Rev.* **2005**, *34*, DOI: 10.1039/b412490a, available on the web.
[12] M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Am. Chem. Soc.* **1991**, *113*, 9360–9361.

[13] M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Org. Chem.* **1992**, *57*, 5643–5649.
[14] M. Inagaki, A. Hatanaka, M. Mimura, J. Hiratake, T. Nishioka, J. Oda, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 111–120.
[15] L. T. Kanerva, K. Rahiala, O. Sundholm, *Biocatalysis* **1994**, *10*, 169–180.
[16] C. Paizs, M. Tosa, C. Majdik, P. Tähtinen, F. D. Irimie, L. T. Kanerva, *Tetrahedron: Asymmetry* **2003**, *14*, 619–627.
[17] C. Paizs, P. Tähtinen, K. Lundell, L. Poppe, F. D. Irimie, L. T. Kanerva, *Tetrahedron: Asymmetry* **2003**, *14*, 1895–1904.
[18] C. Paizs, P. Tähtinen, M. Toşa, C. Majdik, F.-D. Irime, L. T. Kanerva, *Tetrahedron* **2004**, *60*, 10533–10540.
[19] L. Veum, U. Hanefeld, *Tetrahedron: Asymmetry* **2004**, *15*, 3707–3709.
[20] Y. Zhu, L.-R. Yang, Z.-Q. Yao, S. Zhu, P. Cen, *Ann. New York Acad. Sci.* **1998**, *864*, 646–648.
[21] T. Zhang, L. Yang, Z. Zhu, J. Wu, *J. Mol. Catal. B Enzym*. **2002**, *18*, 315–323.
[22] Y.-X. Li, A. J. J. Straathof, U. Hanefeld, *Tetrahedron: Asymmetry* **2002**, *13*, 739–743.
[23] U. Hanefeld, Y. Li, R. A. Sheldon, T. Maschmeyer, *Synlett* **2000**, *12*, 1775–1776.
[24] L. Veum, M. Kuster, S. Telalovic, U. Hanefeld, T. Maschmeyer, *Eur. J. Org. Chem.* **2002**, 1516–1522.
[25] E. Zacharis, B. D. Moore, P. J. Halling, *J. Am. Chem. Soc.* **1997**, *119*, 12396–12397.
[26] N. Fontes, J. Partridge, P. J. Halling, S. Barreiros, *Biotechnol. Bioeng*. **2002**, *77*, 296–305.
[27] A. Basso, L. De Martin, C. Ebert, L. Gardossi, P. Linda, *J. Mol. Catal. B Enzym*. **2000**, *8*, 245–253.
[28] U. Hanefeld, A. J. J. Straathof, J. Heijnen, *J. Mol. Catal. B. Enzym.* **2001**, *11*, 213–218.
[29] N. W. J. T. Heinsman, C. G. P. H. Schroën, A. van der Padt, M. C. R. Franssen, R. M. Boom, K. van't Riet, *Tetrahedron: Asymmetry* **2003**, *14*, 2699–2704.
[30] L. T. Kanerva, O. Sundholm, *J. Chem. Soc. Perkin Trans. 1* **1993**, 2407–2410.
[31] M. Kitamura, M. Tokunaga, R. Noyori, *Tetrahedron* **1993**, *49*, 1853–1860.
[32] Y. N. Belekon, P. Carta, A. V. Gutnov, V. Maleev, M. A. Moskalenko, L. V. Yashkina, N. S. Ikonnikov, N. V. Voskoboev, V. N. Khrustalev, M. North, *Helv. Chim. Acta* **2002**, *85*, 3301–3312.
[33] A. Fishman, M. Zviely, *Tetrahedron: Asymmetry* **1998**, *9*, 107–118.
[34] H. M. R. Hoffmann, Z. M. Ismail, R. Hollweg, A. R. Zein, *Bull. Chem. Soc. Jpn*. **1990**, 63, 1807–1810.
[35] M. Schmidt, S. Herve, N. Klempier, H. Griengl, *Tetrahedron* **1996**, *52*, 7833–7840.
[36] M. Scholl, C. K. Lim, G. C. Fu, *J. Org. Chem.* **1995**, *60*, 6229–623.

