Organic &
Biomolecular
Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 1112

www.rsc.org/obc

PAPER

In Situ Evaluation of Lipase Performances Through Dynamic Asymmetric Cyanohydrin Resolution

Morakot Sakulsombat, Pornrapee Vongvilai and Olof Ramström*

Received 21st September 2010, Accepted 3rd November 2010
DOI: 10.1039/c0ob00759e

A dynamic resolution process based on multiple reversible cyanohydrin formation coupled to lipase-mediated transesterification is demonstrated. The resulting process resulted in the efficient evaluation of complex lipase performances in asymmetric cyanohydrin acylate synthesis. Dynamic systems were generated and resolved *in situ*, and the effects of the reaction conditions could be directly monitored for the overall system. By this concept, the enzyme activity, chemo- and stereoselectivity for all involved substrates could be simultaneously evaluated.

Introduction

Constitutional Dynamic Chemistry (CDC) has emerged as a powerful technique for the synthesis and evaluation of active compounds acting as substrates, inhibitors and drug leads for biological systems, formation of synthetic receptors, and fabrication of adaptive materials.[1–3] This concept is generally based on reversible interconnections between series of molecules, through either covalent or non-covalent bonds, generating dynamic systems under thermodynamic control. Under the influence of external factors, specific constituents can be preferentially selected, and the dynamic systems forced to re-equilibrate at the benefit of the selected species.

We have recently proposed an efficient substrate matching method using bio-catalytic transformation based on CDC. Dynamic reversible systems, using covalent bond formation, were generated and applied to selected target enzymes.[4–6,3m] The best enzyme substrates were thermodynamically generated, and selectively transformed in a kinetically irreversible process until the resolution process reached completion. An absolute advantage of this approach is the efficient screening of new reactions, and substrates showing strong preference for the biological target in one single operation. In these cases, enzymes were employed as resolving agents, and biocatalysts have been recognized as especially useful in many research laboratories, and in the chemical and pharmaceutical industry, for controlled chemical transformations.[7–12] Among all biocatalysts, lipases constitute a family that is commonly used for syntheses targeting a variety of structures, including bioactive,[9] macrocyclic[13] and asymmetric[14] compounds. Lipases are often commercially available, stable at a variety of reaction conditions and can be used in non-aqueous solution. This family of enzymes has also been extensively studied, catalyzing a wide range of substrates with high regio- and stereospecificity.[14–17]

The reversible cyanohydrin reaction, a potent carbon-carbon bond forming reaction, can be generated from the reaction between an aldehyde or ketone and a cyanide source. The resulting cyanohydrin building blocks are synthetically very versatile and can be transformed into a range of functional groups. Furthermore, cyanohydrin derivatives constitute the core structures of many biologically active compounds.[18–20] In these applications, the demand for optically pure cyanohydrins is especially high. There are several synthetic pathways to synthesize chiral cyanohydrin compounds using both synthetic catalysts,[20,21] and biocatalysts.[22–25] In the biocatalytic reactions, lyases (oxynitrilases),[23] and hydrolases (lipases)[24] have been especially used to produce asymmetric cyanohydrin compounds. For example, an attractive enzymatic synthetic method to high conversions and enantiomeric purities of chiral cynanohydrin esters has been developed by Oda and coworkers,[26–29] where dynamic kinetic resolution was adopted to resolve cyanohydrin products in a one-pot system. This method was later developed to increase the product yields and the optical purities, and to obtain larger structural variety of the products.[24,30] These studies have revealed that lipase-mediated resolution of cyanohydrin structures is efficient, but requires careful optimization of all reaction conditions in order to obtain high yields and high enantiomeric purities. Generally, parameters such as reaction solvent, temperature, acylating reagent, cyanide source, additives, enzyme preparation, etc, need to be evaluated for each structure,[31–33] and in many cases one factor can influence the others.[24,30,34] Cross-evaluation of several parameters are thus often necessary. More efficient methods for reaction optimization and screening of lipase preferences at different conditions are thus needed. In the present study, we have addressed this challenge, and applied a dynamic multisubstrate resolution system for the rapid evaluation of lipase performances of cyanohydrin structures (Fig. 1). With this method, a range of potential substrates can be screened simultaneously *in situ* at any given condition, resulting in efficient identification of optimal structures.

Department of Chemistry, KTH-Royal Institute of Technology, Teknikringen 30, S-10044, Stockholm, Sweden. E-mail: ramstrom@kth.se; Fax: (+) 46 8 7912333

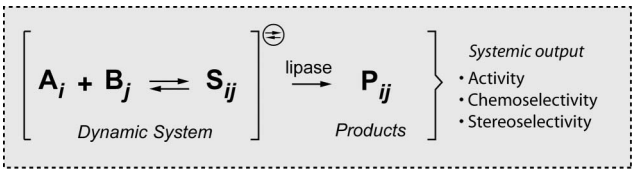
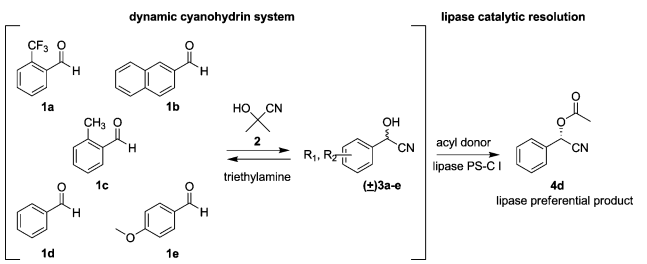


Fig. 1 Dynamic multisubstrate resolution process. A range of substrates (S_{ij}) is formed through dynamic reactions of individual components A_i and B_j, and the resulting system directly resolved by the enzyme to form products P_{ij}. The process yields direct information of the enzyme performance for specific substrates.

Results and Discussion

The dynamic cyanohydrin reaction was initially evaluated, and dynamic cyanohydrin systems were generated according to Scheme 1. Although the reversible cyanohydrin reactions can be generated by different cyanide sources,[23,24,34] one of the most convenient and less harmful methods is using acetone cyanohydrin **2** in the presence of base.[28,29] This mildly results in release of cyanide ion, and the formation of acetone as a by-product. Five different aromatic aldehydes, 2-trifluoromethylbenzaldehyde **1a**, 2-naphthaldehyde **1b**, 2-methylbenzaldehyde **1c**, benzaldehyde **1d,** and 4-methoxybenzaldehyde **1e,** were chosen and investigated in the reversible cyanohydrin reaction. This system was chosen as a prototype in order to demonstrate the efficiency of the process, but can be easily expanded to accommodate a larger collection of aldehydes. In the present case, aromatic aldehydes were exclusively used, resulting in similar reactivities, corresponding stable intermediates, and no generation of any side products. However, aliphatic aldehydes can also be efficiently applied. Different amine bases (pyridine, triethylamine, *N*, *N*-diisopropylethylamine, 4-dimethylaminopyridine) were assessed for generation of the dynamic system, where triethylamine was selected because of rapid cyanide release, no formation of side products, and no interference with the signature signals (α-protons) in the NMR spectra. The reactions proved sufficiently rapid and when equimolar amounts of the aldehydes, acetone cyanohydrin **2** and triethylamine were mixed in chloroform-*d* at room temperature, dynamic cyanohydrin systems were rapidly formed that reached equilibrium within three hours as monitored by ^{1}H-NMR. The amount of base was also varied in order to increase the equilibration time, where one equivalent was found adequate for the dynamic system to equilibrate during the enzymatic reaction.



Scheme 1 Generation of dynamic cyanohydrin system and lipase-mediated asymmetric substrate resolution.

^{1}H-NMR spectroscopy was used to monitor the relative amounts of all components and constituents in the dynamic systems formed. At the conditions used, the equilibrium concentrations of the cyanohydrin species were high (K_A ~ 0.6 M^{-1} for the overall cyanohydrin equilibrium). Fig. 2A shows the α-proton region of the cyanohydrin intermediates (5.40–5.95 ppm) in the dynamic system at equilibrium. As can be seen, the intermediates are present at similar levels, where cyanohydrin **3a** is the most favored species and intermediate **3e** present at the lowest level.

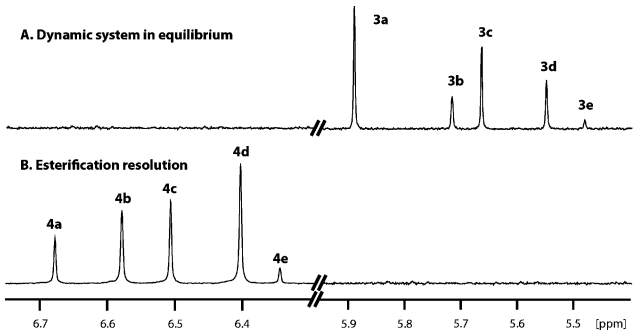
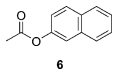


Fig. 2 ^{1}H-NMR spectra (enlarged areas). A. Dynamic cyanohydrin system at equilibrium; B. Lipase-mediated formation of ester products (3 mg PS–C–I, $CDCl_3$, 0 °C).

The enzyme-mediated resolution of the dynamic cyanohydrin system was subsequently explored. Several reaction parameters were addressed including: lipase source and preparation, acylating reagent, reaction solvent, catalyst loading, and reaction temperature. A series of lipases was initially evaluated using the basic conditions developed for the dynamic cyanohydrin system generation. Thus, lipases from porcine pancreas, *Pseudomonas fluorescens*, *Candida rugosa*, *Candida antarctica*, and *Burkholderia cepacia* (previously *Pseudomonas cepacia*), were all probed for the resolution process. Of these, the lipase from *Burkholderia cepacia* proved superior to all others in terms of activity and enantioselectivity and was thus chosen. Although many different lipase preparations are commercially available,[7,14,16,35,36] immobilized lipases are commonly used because of their reported high reactivity, easy handling, and durability at many reaction conditions. These preparations are generally stable for prolonged reaction times, and can be easily filtered off and reused after use.[9] In the present system, three different commercial preparations of the lipase from *Burkholderia cepacia* were tested (Amano PS, PS–C–I, PS–C-II). The lyophilized enzyme (PS), and the immobilized preparation PS–C-II proved inadequate due to sluggish reaction rates, whereas lipase preparation PS–C–I, immobilized on ceramic beads, proved efficient.

The acylating reagent was next addressed, and isopropenyl acetate was first chosen as acyl donor in the lipase-catalyzed resolution. This acylating reagent is commonly used in lipase-mediated acylations because the released acetone by-product is not interfering with the enzyme reaction. In addition, both isopropenyl acetate and acetone can be easily removed after the reactions.[34] In the present case, isopropenyl acetate was applied to the dynamic cyanohydrin system in the presence of lipase preparation PS–C–I at room temperature in chloroform. To reduce the quantity of water in the enzymatic reactions, molecular sieve 4 Å was also added to the systems. The enzymatic reaction was allowed to proceed for 11 days, after which time full conversion was attained. Formation of the resulting ester products **4a–e** (6.30-6.75 ppm) could be easily monitored by ^{1}H-NMR (*cf*. Fig. 2B).

Table 1 Dynamic cyanohydrin resolution with different solvents and acyl donors

entry	Solvent	acyl donor	amount of lipase/mg	reaction time (days)
1	Chloroform	 5	10	11
2	Chloroform	 5	6	14
3	Chloroform	 5	3	20
4	Chloroform	 6	10	7
5	Chloroform	 7	10	7
6	Toluene	 5	10	11
7	Toluene	 5	3	20
8	TBME	 5	10	>30

Reaction conditions: Solutions of the dynamic cyanohydrin system (1 mL) and acyl donor (5 equivalents) were added to the lipase and 10 mg of MS4 Å. The resolution reactions were monitored by ^{1}H NMR spectroscopy until complete conversions.

The resulting NMR patterns were used to estimate the lipase preference factors (F_P), representing the selectivities of the enzyme for the various cyanohydrin substrates. The preference factors were calculated as the relative ratios between the product and the corresponding intermediate, respectively (eqn (1) in Experimental section). The initial resolution systems were performed at ambient temperature using 10 mg of PS–C–I preparation in chloroform. Interestingly, the major intermediate **3a** in the dynamic system was disfavored by the lipase. The resulting preference factors are displayed in Fig. 3A (front row), showing moderate selectivities for intermediates **4b** to **4e** (F_p = 0.2-0.4), compared to the unselected cyanohydrin **4a** (F_p = -0.4). To enhance this selectivity, a range of acyl donors were evaluated at the same conditions in addition to isopropenyl acetate **5** (Table 1): 2-naphthyl acetate **6,** phenyl acetate **7** and its derivatives, 4-chlorophenyl acetate and 4-methoxyphenyl acetate. The results indicate that these acyl donors enhance the rate of the enzymatic reactions, whereas the lipase substrate selectivity remained unchanged. Thus, full conversions were attained within 7 days (Table 1, entries 4 and 5).

Then, a variety of solvents were screened in the enzymatic reaction in addition to chloroform: toluene, *tert*-butyl methyl ether, diethyl ether, acetonitrile, dimethyl sulfoxide and dimethyl formamide. Table 1 showed rate of reactions from different solvents providing the fastest lipase resolutions (entries 1, 6 and 8). The reactions using toluene as solvent were completed within the same reaction time as chloroform, and also showed similar preference factors. However, when more polar solvents were used as reaction solvents, the lipase-catalyzed reactions proceeded at a considerably lower rate. For both these parameters, acylating reagent and solvent, the reaction rates proved however the main difference whereas the selectivity patterns remained similar.

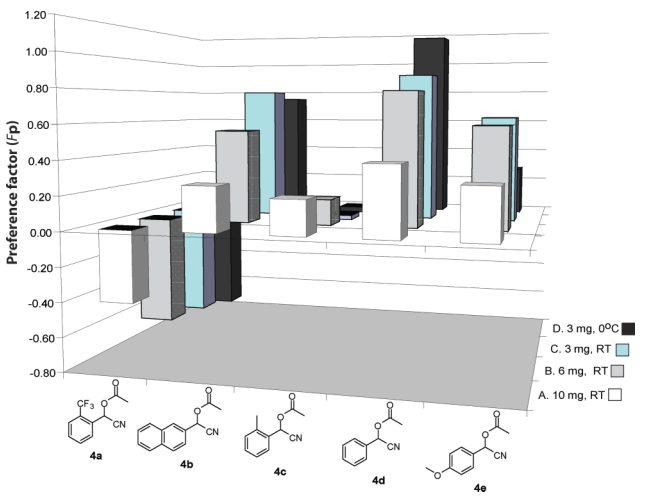


Fig. 3 Substrate preference factors of dynamic cyanohydrin system using A. 10 mg lipase at RT; B. 6 mg lipase at RT; C. 3 mg lipase at RT and D. 3 mg lipase at 0 °C.

The catalyst loading in the process was subsequently evaluated. The enzyme preparation was gradually decreased from 10 mg to 3 mg per sample in order to enhance the enzyme selectivity effects. As expected, the reactions with lower amounts of enzyme (3–6 mg) resulted in reduced reaction rates, 20 and 14 days, respectively,

compared to the higher amount (Table 1, entries 1–3 and 6–7). The ratios of the formed ester products **4a–e** were monitored and the substrate preference factors calculated (Fig. 3B-C). As can be seen, the substrate selectivity increased, the ester product **4d** emerged as the most amplified product. The preference factor for product **4d** thus increased from 0.4 to 0.9 with decreasing catalyst loading to 3 mg per sample. In contrast, the reverse trend was observed for cyanohydrin intermediates **3a** (F_P value; from –0.4 to –0.6) and **3c** (F_P value; from 0.2 to 0), which were increasingly disfavored. These results indicate that careful control of the amount of lipase in the resolution process influences the resulting selectivities. The dynamic competition system could however be efficiently used to reveal the selectivities for the individual cyanohydrin intermediates at these conditions.

The temperature dependence of enzymatic reactions has been studied for many biocatalysts. The rigidity of proteins increases at lower temperature, and this has been used as an advantage to enhance the lipase specificity at lower temperature.[33,37,38] This effect was also probed in the present systems. The dynamic cyanohydrin systems based on 3 mg of enzyme per sample were thus applied to lipase resolution at 0 °C. Because of the lower reaction temperature, the enzymatic resolution was retarded and preceded to completion within 40 days. For these extended reaction times, it can be noted that the enzyme preparation is very stable, and the activity remains essentially unchanged throughout the process.[31] The intermediate ratios of the cyanohydrins **3a–e** were similar to the starting ratios prior to lipase resolution. This showed that the dynamic cyanohydrin system was in equilibrium during the entire resolution process also at lower temperature. The relative amounts of the resulting ester products **4a–e** after completion of the enzymatic reaction is shown in Fig. 2B. The lipase substrate selectivity was obviously enhanced, resulting in different product ratios compared to ambient temperature. The ester product **4d** was in this case preferentially selected ($F_P = 1.2$) and amplified from the dynamic cyanohydrin system, while ester products **4a** and **4c** remained deselected by the lipase catalytic resolution. Interestingly, the lipase also showed lower preference for cyanohydrin intermediates **3b** ($F_P = 0.7$) and **3e** ($F_P = 0.3$) at this temperature. Even lower reaction temperature, at –18 °C was also applied to the dynamic enzymatic resolution system, but the reaction was in this case extremely slow and was stopped at <50% conversion. However, the results at this conversion indicated the same trend, and higher substrate selectivity was recorded. The preferential factor of ester product **4d** was enhanced to 1.7, where ester products **4a** and **4c** were disfavored ($F_p = –0.8$ and –0.36, respectively). The ester product **4b** and **4e** were slightly preferred by lipase ($F_p = 0.8$ and 0.5, respectively). For the first system variations, the specific acyl donor and the reaction solvent, the selectivities did not change significantly, mainly affecting the reaction rates. However, decreasing the catalyst loading and lowering the reaction temperature played crucial role in the lipase substrate selectivity. These strategies decreased the rate of the lipase resolution reaction, while simultaneously enhancing the lipase substrate selectivity. The trend can be easily monitored from the preference factors, where selection of the intermediate **3d** was gradually improved. At the same time, cyanohydrin intermediates **3a** and **3c** were increasingly disfavored.

To explore the lipase enantioselectivity, the ester products from the dynamic resolution process were monitored by chiral chromatography. Because of the acidity of the α–protons of the product esters, the enantiomeric purities were sensitive to the strength and amount of base in the reaction.[28–30,34] In the present systems, one equivalent of triethylamine was used to force the reversible reactions to reach equilibrium, especially at low temperature, and this affected the resulting enantiomeric excesses of the formed product esters **4a–e**. It should be noted that this can be circumvented for singular DKR systems, where the base concentration can be reduced. However, although the ester products were racemized by base in the reaction to some extent, the residual enantiomeric excesses could still be monitored. Thus, after completion of the enzymatic resolutions processes, the enantiomeric excesses of all ester products **4a–e** were monitored by chiral HPLC. Since the ester products and starting aldehydes showed similar polarities, separation of the crude reaction mixtures proved however difficult. To overcome this obstacle, aldehydes **1a–e** were converted to their corresponding bisulfite adducts, which were subsequently filtered off leaving ester products **4a–e** well-resolved (see Supporting Information). The bisulfite reaction *per se* did not have any effect on the enantiomeric excesses. The results indicate strong lipase enantioselectivity of the ester products (Table 2), and all ester products except the disfavored product **4a** were asymmetrically resolved by the lipase transesterification process. Interestingly, the highest enantiomeric ratio of the ester products was recorded for ester product **4e** (83% *ee*). This indicated that the lipase-catalyzed reaction is a powerful tool to resolve optically active cyanohydrin ester products. Although not affecting the overall product ratios, the solvent effect proved important for the enantioselectivity, and when the systems were evaluated in toluene, lower enantioselectivities were recorded compared to chloroform.

Table 2 Effect of solvents on enantiomeric excess of cyanohydrin ester products **4a–e**

		% enantiomeric excess[a]	
entry	product	toluene	chloroform
1	**4a**	0	2
2	**4b**	20	30
3	**4c**	24	36
4	**4d**	34	54
5	**4e**	68	83

[a] The enantiomeric excess of ester products from lipase-mediated resolution was analyzed by HPLC analysis at 200 nm using combination of CPS cyano and OD-H chiral columns; eluent: 2-propanol and hexane (1/99; v/v).

Conclusion

A dynamic resolution process has been developed, resulting in efficient evaluation of lipase performances in asymmetric cyanohydrin synthesis. Prototype dynamic cyanohydrin systems composed of different cyanohydrin intermediates were generated under thermodynamic control and successfully resolved to the corresponding esters under kinetic control. The catalytic lipase resolution process could be applied to the dynamic system, and efficiently used to identify small differences in substrate selectivity. In the present system, one of the ester products (**4d** was identified as the preferred substrate for the lipase from *Burkholderia cepacia*. This preference could also be enhanced from reducing the catalyst

loading and the reaction temperature, resulting in significant amplification compared to the cyanohydrin equilibrium ratio. The enantioselectivity of the lipase could also be easily monitored, where all selected cyanohydrins proved enantiomerically enriched, whereas the deselected species proved less optically resolved. The developed method can be easily expanded to encompass considerably larger collections of cyanohydrins, and is particularly useful for rapid evaluation of lipase selectivities. The dynamic process also leads to facilitated analysis of complex dependencies between reaction parameters, where small differences in selectivity can be easily discerned.

Experimental Section

General

Reagents were purchased from Sigma–Aldrich, Lancaster and Apollo and used as received. Amano Lipase PS–C–I from *Burkholderia cepacia* (Aldrich 534897). ^{1}H and ^{13}C NMR data were recorded on a Bruker Avance 400 spectrometer at 400 (100) MHz and/or a Bruker Avance DMX 500 at 500 (125) MHz, respectively. Chemical shifts are reported as δ values (ppm) with $CDCl_3$ (^{1}H NMR δ 7.26, ^{13}C NMR δ 77.16) as internal standard. J values are given in Hertz (Hz). ESI-Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX mass spectrometer. Analytical high performance liquid chromatography (HPLC) with combinations of cyano and chiral stationary phases was performed on HP-Agilent 1110 Series controller, using ThermoQuest cyano (4.6 × 150 mm) and Daicel Chiralpak OD-H (4.6 xd7; 250 mm, μm) columns, respectively. Solvents for HPLC use were of spectrometric grade. Thin layer chromatography (TLC) was performed on precoated Polygram® SIL G/UV 254 silica plates (0.20 mm, Macherey–Nagel), visualized with UV-detection. Flash column chromatography was performed on silica gel 60, 0.040-0.063 mm (SDS).

General procedure for dynamic cyanohydrin system generation and lipase-mediated screening

The specific amount of lipase preparation and ground 4 Å molecular sieves (10 mg) were weighed in a 2 mL screw-cap vial and dried under vacuum overnight. The dynamic cyanohydrin system, generated by five different aromatic aldehydes (**1a** to **1e**, 0.1 mmol each), acetone cyanohydrin (9.2 μL, 0.1 mmol) and dried triethylamine (14 μL, 0.1 mmol), in the specified dry solvent (1.0 mL), was generated at room temperature overnight. The acylating reagent (0.5 mmol) was added, and the resulting solution added to the reaction vial under N_2 atmosphere. The reaction was performed without stirring at the specific temperature and monitored by ^{1}H NMR spectroscopy until complete. The reaction mixture was filtrated and washed with saturated ammonium chloride solution and saturated sodium chloride solution. The organic layer was dried over magnesium sulfate and evaporated in *vacuo*. For chiral analysis of esters **4a** to **4e**,[39] the resulting reaction mixture was dissolved in ethanol (2 mL). Then, sodium metabisulfite solution (5 mL, sodium metabisulfite 1.6 g in water 10 mL) was added and the reaction was kept in refrigerator for overnight. The precipitate was filtered off and the filtrate extracted with hexane three times. The combined organic layer was washed with sodium chloride solution and dried over magnesium sulfate. The crude reaction was evaporated in *vacuo*. The mixture of ester products was analyzed by connection of ThermoQuest cyano and OD-H chiral HPLC in order to obtain enantiomeric excesses of individual ester product.

General procedure for synthesis of racemic *O*-acetylcyanohydrins[21]

Potassium cyanide (2.5 g, 39.2 mmol), *tert*-butanol (1.0 mL, 10.3 mmol) and water (0.1 mL, 4.4 mmol) were stirred in dichloromethane (20 mL) at room temperature. The aldehyde (9.8 mmol) and acetic anhydride (4.0 g, 39.2 mmol) were added and the reaction mixture was stirred at room temperature for two days. The crude reaction was filtered and washed with dichloromethane. The combined organic layers were evaporated in vacuo. The residue was purified by column chromatography using the mixture of ethyl acetate and hexane as eluent (1 : 4, v/v).

Cyano(2-(trifluoromethyl)phenyl)methyl acetate 4a[30b]

Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 2.18 (s, 3 H, CH_3), 6.68 (s, 1 H, CH), 7.61 (t, J = 7.5 Hz, 1 H, CH), 7.71 (t, J = 7.5 Hz, 1 H, CH), 7.76 (d, J = 8.0 Hz, 1 H, CH), 7.93 (d, J = 8.0 Hz, 1 H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 20.3, 59.4, 115.6, 123.6 (q, J_{CF} = 274 Hz), 126.9 (q, J_{CF} = 5.7 Hz), 128.5 (q, J_{CF} = 31.6 Hz), 129.9, 130.3, 130.8, 133.0, 168.5. ESI-Mass (positive mode) m/z: found 243.2; calcd for $(M + H)^+$ 243.0. Chiral HPLC (cyano and chiralpak OD-H); 0.2 mL min^{-1}, 2-propanol: hexane, 1 : 99, detection 200 nm; t_R 57.6 min, 66.1 min.

Cyano(naphthalen-2-yl)methyl acetate 4b[26]

Yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 2.19 (s, 3H, CH_3), 6.59 (s, 1H, CH), 7.54-7.59 (m, 3H, CH), 7.86-7.94 (m, 3H, CH), 8.02 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 20.6, 63.2, 116.3, 124.4, 127. 2, 127.7, 127.9, 128.1, 128.5, 129.1, 129.6, 133.0, 134.0, 169.1. ESI-Mass (positive mode) m/z: found 225.1; calcd for $(M + H)^+$ 225.0. Chiral HPLC (cyano and chiralpak OD-H); 0.2 mL min^{-1}, 2-propanol: hexane, 1 : 99, detection 200 nm; t_R 162.0 min, 176.9 min.

Cyano(*o*-tolyl)methyl acetate 4c[40]

Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 2.17 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.51 (s, 1H, CH), 7.25 (d, J = 7 Hz, 1H, CH), 7.29 (t, J = 7 Hz, 1H, CH), 7.36 (t, J = 7.5 Hz, 1H, CH), 7.56 (d, J = 8.5 Hz, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 19.0, 20.5, 61.1, 116.1, 126.9, 128.6, 130.0, 130.6, 131.4, 136.7, 168.9. ESI-Mass (positive mode) m/z: found 189.0; calcd for $(M + H)^+$ 189.0. Chiral HPLC (cyano and chiralpak OD-H); 0.2 mL min^{-1}, 2-propanol: hexane, 1 : 99, detection 200 nm; t_R 74.8 min, 76.8 min.

Cyano(phenyl)methyl acetate 4d[29]

Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 2.17 (s, 3H, CH_3), 6.41 (s, 1H, CH), 7.42-7.48 (m, 3H, CH), 7.49-7.55 (m, 2H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 20.6, 63.0, 116.3, 128.0, 129.4, 130.5, 131.9, 169.0. ESI-Mass (positive mode) m/z: found 175.0; calcd for $(M + H)^+$ 175.0. Chiral HPLC (cyano

and chiralpak OD-H); 0.2 mL min^{-1}, 2-propanol: hexane, 1 : 99, detection 200 nm; t_R 82.3 min, 89.1 min.

Cyano(4-methoxyphenyl)methyl acetate 4e[40]

Colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 2.14 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 6.35 (s, 1H, CH), 6.95 (d, J = 9 Hz, 2H, CH), 7.45 (d, J = 9 Hz, 2H, CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C) δ 20.7, 55.6, 62.7, 114.7, 116.5, 124.0, 129.7, 161.3, 169.1. ESI-Mass (positive mode) m/z: found 205.0; calcd for $(M + H)^+$ 205.0. Chiral HPLC (cyano and chiralpak OD-H); 0.2 mL min^{-1}, 2-propanol: hexane, 1 : 99, detection 200 nm; t_R 111.8 min, 125.4 min.

Preference factor (F_p)

The preference factors were estimated according to eqn (1):

$$F_p = [C_r(\text{ester})\text{-}C_r(\text{intermediate})]/C_r(\text{intermediate}) \quad (1)$$

where C_r(intermediate) and C_r(ester) are the relative concentrations of the intermediate cyanohydrin and the product ester, respectively.

Acknowledgements

This work was supported by the Swedish Research Council, the Royal Institute of Technology, and the European Commission (contract no. MRTN-CT-2006-035614).

Notes and references

1 For books see: (*a*) *Dynamic Combinatorial Chemistry*, ed. J. Reek and S. Otto, Wiley-VCH, Weinheim, 2010; (*b*) *Dynamic Combinatorial Chemistry – in drug discovery, bioorganic chemistry, and materials science*, ed. B. J. Miller, Wiley, New York, 2009.
2 For reviews, see: (*a*) J.-M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151–160; (*b*) B. de Bruin, P. Hauwert and J. N. H. Reek, *Angew. Chem. Int. Ed.*, 2006, **45**, 2660–2663; (*c*) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711; (*d*) M. Crego-Calama, D. N. Reinhoudt and M. G. J. ten Cate, *Top. Curr. Chem.*, 2005, **249**, 285–316; (*e*) O. Ramström and J.-M. Lehn, *Nat. Rev. Drug Discovery*, 2002, **1**, 26–36; (*f*) O. Ramström, T. Bunyapaiboonsri, S. Lohmann and J.-M. Lehn, *Biochim. Biophys. Acta*, 2002, **1572**, 178–186.
3 For recent reports, see: (*a*) R. Caraballo, H. Dong, J. P. Ribeiro, J. Jiménez-Barbero and O. Ramström, *Angew. Chem. Int. Ed.*, 2010, **49**, 589–593; (*b*) P. Besenius, P. A. G. Cormack, R. F. Ludlow, S. Otto and D. C. Sijbren Sherrington, *Org. Biomol. Chem.*, 2010, **8**, 2414–2418; (*c*) H. Otsuka, S. Nagano, Y. Kobashi, T. Maeda and A. Takahara, *Chem. Commun.*, 2010, 1150–1152; (*d*) V. Saggiomo and U. Luening, *Chem. Commun.*, 2009, 3711–3713; (*e*) S. Rochat and K. Severin, *J. Comb. Chem.*, 2010, **12**, 595–599; (*f*) S. Ghosh, L. A. Ingerman, A. G. Frye, S. J. Lee, M. R. Gagné and M. L. Waters, *Org. Lett.*, 2010, **12**, 1860–1863; (*g*) J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J.-P. Peyralans and S. Otto, *Science*, 2010, **327**, 1502–1506; (*h*) L. Tauk, A. P. Schröder, G. Decher and N. Giuseppone, *Nature Chem.*, 2010, **1**, 649–656; (*i*) V. C. Allen, C. C. Robertson, S. M. Turega and D. Philp, *Org. Lett.*, 2010, **12**, 920–1923; (*j*) A. Herrmann, N. Giuseppone and J.-M. Lehn, *Chem. Eur. J.*, 2009, **15**, 117–124; (*k*) H. Y. Au-Yeung, G. D. Pantos and J. K. M. Sanders, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 10466–10470; (*l*) P. Mal, B. Breiner, K. Rissanen and J. R. Nitschke, *Science*, 2009, **324**, 1697–1699; (*m*) P. Vongvilai and O. Ramström, *J. Am. Chem. Soc.*, 2009, **131**, 14419–14425; (*n*) M. Barboiu, F. Dumitru, Y.-M. Legrand, E. Petit and A. Van Der Lee, *Chem. Commun.*, 2009, 2192–2194.
4 R. Larsson, Z. Pei and O. Ramström, *Angew. Chem., Int. Ed.*, 2004, **43**, 3716–3718.
5 R. Larsson and O. Ramström, *Eur. J. Org. Chem.*, 2005, 285–291.
6 P. Vongvilai, M. Angelin, R. Larsson and O. Ramström, *Angew. Chem., Int. Ed.*, 2007, **46**, 948–950.
7 E. M. Anderson, K. M. Larsson and O. Kirk, *Biocatal. Biotransform.*, 1998, **16**, 181–204.
8 M. Ikunaka, *Chem. Eur. J.*, 2003, **9**, 378–388.
9 J. M. Palomo, *Curr. Bioact. Compd.*, 2008, **4**, 126–138.
10 M. T. Reetz, *Angew. Chem., Int. Ed.*, 2002, **41**, 1335–1338.
11 M. T. Reetz, *Adv. Catal.*, 2006, **49**, 1–69.
12 R. Wohlgemuth, *New Biotechnol.*, 2009, **25**, 204–213.
13 F. Bracher and B. Schulte, *Nat. Prod. Res.*, 2003, **17**, 293–299.
14 A. Ghanem, *Tetrahedron*, 2007, **63**, 1721–1754.
15 T. Ema, *Tetrahedron: Asymmetry*, 2004, **15**, 2765–2770.
16 E. Fukusaki and S. Satoda, *J. Mol. Catal. B: Enzym.*, 1997, **2**, 257–269.
17 F. Theil, *Chem. Rev.*, 1995, **95**, 2203–2227.
18 M. Avi, R. Gaiberger, S. Feichtenhofer and H. Griengl, *Tetrahedron*, 2009, **65**, 5418–5426.
19 F. Effenberger, S. Foerster and C. Kobler, *Biocatal. Pharm. Biotechnol. Ind.*, 2007, **677-698**, 671.
20 M. North, D. L. Usanov and C. Young, *Chem. Rev.*, 2008, **108**, 5146–5226.
21 Y. N. Belokon, A. J. Blacker, L. A. Clutterbuck, D. Hogg, M. North and C. Reeve, *Eur. J. Org. Chem.*, 2006, 4609–4617.
22 G. Seoane, *Curr. Org. Chem.*, 2000, **4**, 283–304.
23 H. Griengl, H. Schwab and M. Fechter, *Trends Biotechnol.*, 2000, **18**, 252–256.
24 J. Holt and U. Hanefeld, *Curr. Org. Synth.*, 2009, **6**, 15–37.
25 D. V. Johnson, A. A. Zabelinskaja-Mackova and H. Griengl, *Curr. Opin. Chem. Biol.*, 2000, **4**, 103–109.
26 M. Inagaki, A. Hatanaka, M. Mimura, J. Hiratake, T. Nishioka and J. Oda, *Bull. Chem. Soc. Jpn.*, 1992, **65**, 111–120.
27 M. Inagaki, J. Hiratake, T. Nishioka and J. Oda, *Bull. Inst. Chem. Res., Kyoto Univ.*, 1989, **67**, 132–135.
28 M. Inagaki, J. Hiratake, T. Nishioka and J. Oda, *J. Am. Chem. Soc.*, 1991, **113**, 9360–9361.
29 M. Inagaki, J. Hiratake, T. Nishioka and J. Oda, *J. Org. Chem.*, 1992, **57**, 5643–5649.
30 (*a*) Q. Xu, X. Geng and P. Chen, *Tetrahedron Lett.*, 2008, **49**, 6440–6441; (*b*) T. Sakai, K. Wang and T. Ema, *Tetrahedron*, 2008, **64**, 2178–2183.
31 T. Ema, S. Maeno, Y. Takaya, T. Sakai and M. Utaka, *Tetrahedron: Asymmetry*, 1996, **7**, 625–628.
32 M. Persson, D. Costes, E. Wehtje and P. Adlercreutz, *Enzyme Microb. Technol.*, 2002, **30**, 916–923.
33 T. Sakai, *Tetrahedron: Asymmetry*, 2004, **15**, 2749–2756.
34 L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer and U. Hanefeld, *Adv. Synth. Catal.*, 2005, **347**, 1015–1021.
35 P. Dominguez de Maria, C. Carboni-Oerlemans, B. Tuin, G. Bargeman, A. Van Der Meer and R. van Gemert, *J. Mol. Catal. B: Enzym.*, 2005, **37**, 36–46.
36 K. Laumen, M. Kittelmann and O. Ghisalba, *J. Mol. Catal. B: Enzym.*, 2002, **19-20**, 55–66.
37 T. Sakai, K. Hayashi, F. Yano, M. Takami, M. Ino, T. Korenaga and T. Ema, *Bull. Chem. Soc. Jpn.*, 2003, **76**, 1441–1446.
38 T. Sakai, T. Kishimoto, Y. Tanaka, T. Ema and M. Utaka, *Tetrahedron Lett.*, 1998, **39**, 7881–7884.
39 H. Göker, S. Özden, S. Yildiz and D. W. Boykin, *Eur. J. Med. Chem*, 2005, **40**, 1062–1069.
40 P.-T. Lee and C. Chen, *Tetrahedron: Asymmetry*, 2005, **16**, 2704–2710.