DOI: 10.1002/adsc.200700053

Hydrolase-Catalysed Preparation of Chiral α,α-Disubstituted Cyanohydrin Acetates

Jarle Holt,[a] Isabel W. C. E. Arends,[a] Adriaan J. Minnaard,[b] and Ulf Hanefeld[a,*]

[a] Biokatalyse en Organische Chemie, Technische Universiteit Delft, Julianalaan 136, 2628 BL Delft, The Netherlands
Fax: (+31)-(0)15-278-1415; e-mail: u.hanefeld@tudelft.nl
[b] Synthetisch Organische Chemie, Stratingh Instituut, Rijksuniversiteit Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Received: January 24, 2007

Dedicated to Prof. Dr. Liisa T. Kanerva on the occasion of her 60th birthday.

Abstract: The enzymatic hydrolysis of esters of tertiary alcohols has long been a challenge. In particular their kinetic resolutions have virtually not been addressed. Here we describe the successful kinetic resolution of α,α-disubstituted cyanohydrin acetates, a type of protected tertiary alcohols that form particularly interesting building blocks in organic synthesis. Utilising Subtilisin A the hydrolysis reaction was (*S*)-selective, while *Candida rugosa* lipase was (*R*)-selective. With these commercially available enzymes both enantiomers of the α,α-disubstituted cyanohydrin acetates are now accessible.

Keywords: asymmetric catalysis; cyanohydrins; enzyme catalysis; hydrolases; kinetic resolution; tertiary alcohols

Cyanohydrins are versatile building blocks in organic synthesis and in the fine chemical industry. Their enantioselective, catalytic preparation has attracted much attention and both chemical and enzymatic approaches have been developed.[1–4] In most cases the (bio)catalytic methods focus on aldehydes as starting materials, since stereodifferentiation of these prochiral molecules is relatively easy. When starting from ketones the induction of chirality becomes considerably more difficult. In addition, the reaction equilibrium is less favourable since the product, an α,α-disubstituted cyanohydrin is sterically congested, indeed it is a tertiary alcohol. When preparing them enantiopure by HCN addition, a large excess thereof needs to be used, independent of whether the reaction was catalysed chemically or with an enzyme.[1–4] Chemical approaches with activated cyanides such as TMSCN have been described in which the product is immediately protected, ensuring a shift of the equilibrium.[3] Similarly, acid anhydrides can be added for this purpose.[5] Attempts in this direction have also been performed in the enantioselective hydroxynitrile lyase-catalysed reactions, but the success is still limited to few substrates.[6]

A different approach is to view these α,α-disubstituted cyanohydrins as tertiary alcohols. Their racemic esters are straightforward to prepare and a kinetic resolution should allow an uncomplicated access to the chiral cyanohydrin. However, tertiary alcohols and their esters are notoriously problematic substrates for hydrolases, the enzymes of choice for a kinetic resolution (Figure 1).[7–12] Only few examples of the

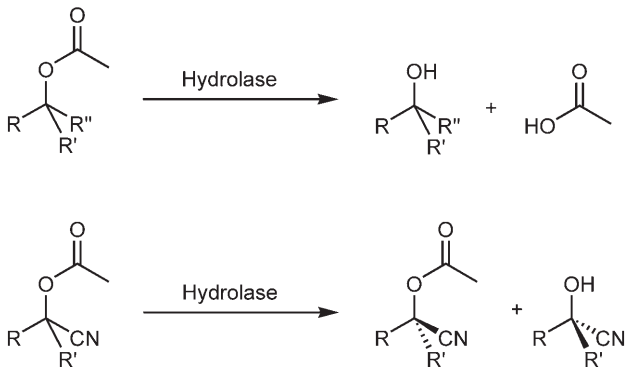
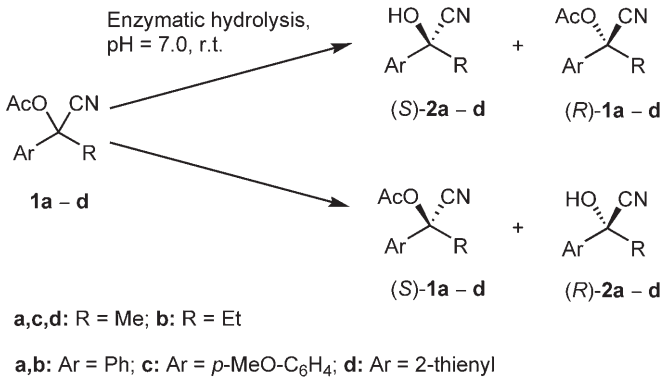


Figure 1. The enzymatic hydrolysis of tertiary alcohols – i.e., of α,α-disubstituted cyanohydrins – is a challenge.

successful hydrolysis of these esters are known, enantioselective conversions are even fewer and the application for α,α-disubstituted cyanohydrins is very limited. Only two non-commercial crude enzymes were described to perform this reaction with limited yields and *ee*s[13–15] and just one successful example (α-trifluoromethyl-α-ethyl cyanohydrin acetate) for *Candida rugosa* lipase (CRL) is known.[16] Taking this as a starting point we set out for an extensive screening of hydrolases for the kinetic resolution of α,α-disubstituted cyanohydrin acetates.

Given the rather limited knowledge about the ability of hydrolases to catalyse the enantioselective hydrolysis of α,α-disubstituted cyanohydrin esters, a general screening of lipases, esterases and proteases was performed [*Candida rugosa* lipase (CRL), *Rhizomucor miehei* lipase, *Burkholderia cepacia* lipase that is also known as *Pseudomonas cepacia* lipase immobilised on ceramic particles (BCL), *Candida antarctica* lipase A (Novozyme 735, liquid preparation), *Candida antarctica* lipase A (immobilised on Celite R-633), *Candida antarctica* lipase B (Novozyme 435), porcine pancreas lipase, pig liver esterase, α-chymotrypsin, Subtilisin A (SubA; type VIII, from *Bacillus licheniformis*)]. To ensure comparability, the same amount of units was used for all enzymes. As substrates the readily prepared acetates **1a–d** were employed in a pH stat-controlled reaction (Scheme 1). Under these



Scheme 1. General conditions for the screening of hydrolytic enzymes.

conditions the conversions can be followed by the addition of base. The unreactive enantiomer of **1a–d** can readily be isolated while the released cyanohydrins **2a–d** tend to liberate HCN and the corresponding ketone.[17,18]

Three significant activities were found (Table 1). In line with earlier results, CRL is capable of hydrolysing tertiary alcohols, including those that have a carbon-carbon triple bond instead of a nitrile group.[8,10,12] As observed earlier for α-trifluoromethyl-α-ethyl cyanohydrin acetate[16] CRL selectively hydrolyses (*R*)-**1a** and (*R*)-**1d** with reasonable enantioselectivity ($E \geq 8$). Surprisingly, the other compounds were not substrates for CRL. BCL only displayed activity for **1a**; similar to CRL it is modestly *R*-selective. This activity was unexpected, since earlier studies with structurally related compounds (C≡C in place of a nitrile group)[8,12] revealed no activity. Against predictions CAL-A did not show any activity. Neither when immobilised on Celite, nor when used as a liquid preparation could any activity be detected for this enzyme. This is all the more surprising since

Table 1. Enzymatic hydrolysis of **1a–d**, only reactions where activity was observed are given.[a]

	Enzyme	Time	Conv. **1**	*ee* **1**	**E**
1a	CRL	96 h	60%	91% (*S*)	13
1a	BCL	48 h	44%	47% (*S*)	6
1a	SubA	72 h	55%	88% (*R*)	18
1b	SubA	72 h	41%	64% (*R*)	44
1c	SubA	20 h	50%	90% (*R*)	58
1d	CRL	20 h	61%	54% (*S*)	8
1d	SubA	20 h	64%	87% (*R*)	7

[a] *Conditions:* 200 U enzyme, 1 mmol substrate, 10 mL of 10 mM phosphate buffer at pH 7.0 and 25 °C, for details of the reaction conditions and analysis, see Experimental Section. The cyanohydrins **2** that are released during the reactions decompose under the reaction conditions to the corresponding ketones. The cyanohydrin acetate of 2-acetylfuran was not converted by any of the enzymes.

CAL-A is known for its activity towards the esters of tertiary alcohols, in particular 2-phenylbut-3-yn-2-ol acetate. This molecule differs from **1a** only by a C≡C in the position of the nitrile group, indicating the importance of electronic effects for the enzyme activity.[7,10,11]

The most notable result is the significant activity and selectivity of the protease Subtilisin A (SubA) for all substrates **1a–d**. With an enantioselectivity as high as $E = 58$ for **1c**, the stereoselectivity of this enzyme is remarkable. In addition, SubA displays (*S*)-enantioselectivity, the opposite to that of lipases CRL and BCL. It thus complements them, allowing for a flexible approach towards both stereoisomers. This stereocomplementarity of SubA to lipases is already known for secondary alcohols.[19]

In summary, a new hydrolase-based enantioselective approach towards esters of α,α-disubstituted cyanohydrins has been developed, that forms the basis for a new approach towards their enantioselective preparation. SubA stereoselectively hydrolyses the (*S*)-enantiomer of their acetates **1a–d**, while CRL and BCL display activity and selectivity for the (*R*)-enantiomers.

Experimental Section

Materials and Methods

Trimethylsilyl cyanide (Fluka), *N*-methylmorpholine *N*-oxide (97%, Sigma–Aldrich), dichloromethane (anhydrous, 99.8%, Sigma–Aldrich), hydrofluoric acid (48%, Sigma–Aldrich), acetic anhydride (99+%, Acros), pyridine (anhydrous, 99.8%, Sigma–Aldrich), *Candida rugosa* lipase (type VII, 1170 U/mg, Sigma–Aldrich), Subtilisin A (type VIII, from *Bacillus licheniformis*, crystallised and lyophilised, 12 U/mg, Sigma–Aldrich), *Burkholderia cepacia* lipase formerly known as *Pseudomonas cepacia* lipase (immobilised on Ce-

ramic particles, 15156 U/g, Fluka), *Candida antarctica* lipase A (Novozyme 735, liquid preparation), *Candida antarctica* lipase A (Novozyme 735, immobilised on Celite Bio-Catalyst Carrier R-633 from World Minerals according to the literature[20]), *Candida antarctica* lipase B (Novozyme 435), α-chymotrypsin (Sigma–Aldrich), pig liver esterase (Sigma–Aldrich), porcine pancreas lipase (type II, Sigma–Aldrich), *Rhizomucor miehei* lipase (Novo Industri A/S).

All work was performed in the presence of an HCN detector. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 400 (400 MHz and 100 MHz, respectively) or a Varian Unity Inova 300 (300 MHz and 75 MHz, respectively) instrument. Chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Coupling constants *J* are expressed in Hertz (Hz). Mass spectra were determined with a Shimadzu GC-2010 Gas Chromatograph coupled to a Shimadzu GCMS-QP2010S Gas Chromatographic Mass Spectrometer. Optical rotations were obtained using a Perkin–Elmer 343 polarimeter (wavelength 589 nm). The enzyme activity was determined with tributyrin according to a general procedure from the literature.[21] Enzymatic hydrolysis reactions were performed on an automated Dosimat pH-stat from Metrohm. The amount of 0.1 M NaOH is equivalent to the conversion. Enantiomeric purity was determined by GC using an enantioselective β-cyclodextrin column (CP-Chirasil-Dex CB 25 m × 0.32 mm) using a Shimadzu Gas Chromatograph GC-17A equipped with an FID detector and a Shimadzu Auto-injector AOC-20i, using He with a linear gas velocity of 75 cm s^{-1} as the carrier gas. All analyses were performed isothermally. The temperature programs and retention times are: **1a**: 110 °C (20 min): 12.03 min (*S*) and 14.03 min (*R*); **1b**: 110 °C (20 min): 16.18 min (*S*) and 17.30 min (*R*); **1c**: 150 °C (10 min): 7.66 min (*S*) and 8.04 min (*R*); **1d**: 130 °C (10 min): 4.93 min (*S*) and 5.07 min (*R*).

The absolute configuration of **1a** was determined by comparison with optical rotation[22] and elution order of the enantiomers (as their TMS ethers) by enantioselective GC.[23,24] Conversion of the enantiopure acetate to its TMS ether was performed according to a procedure from the literature.[25]

Racemic cyanohydrin acetates were prepared by silylcyanation, deprotection and acylation according to the literature,[26–28] the spectroscopic data are in accordance with the literature:

1a[22]: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.53–7.51 (d, 2H, ArH, *J* = 7.5 Hz), 7.38–7.41 (m, 3H, ArH), 2.14, (s, 3H, CH_3), 1.98 (s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 168.3 (C=O), 138.2 (C≡N), 129.2, 128.9 (×2), 124.2 (×2), 118.2, 73.2, 29.7, 20.9; MS: *m/z* = 189 (M^+), 175, 166, 147, 130, 120, 103, 91, 77, 63, 43.

1b: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42–7.46 (d, 2H, ArH, *J* = 7.54 Hz), 7.26–7.40 (m, 3H, ArH), 2.29 (dq, 1H, CH_2, *J* = 7.25 Hz), 2.13 (s, 3H, CH_3), 2.09 (dq, 1H, CH_2, *J* = 7.25 Hz), 1.03 (t, 3H, CH_3, *J* = 7.35 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 168.3 (C=O), 137.0 (C≡N), 129.0, 128.8 (×2), 124.8 (×2), 117.2, 78.0, 35.8, 20.9, 8.4; MS: *m/z* = 204 (M^++1), 191, 175, 161, 144, 135, 117, 105, 84, 75, 61, 40; anal. calcd. for $C_{12}H_{13}NO_2$ (203.25): C 70.92, H 6.45, N 6.89; found: C 70.5, H 6.1, N 6.7.

1c: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.47 (m, 2H, ArH), 6.89–6.94 (m, 2H, ArH), 3.80 (s, 3H, OCH_3), 2.11 (s, 3H, CH_3), 1.98 (s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$): δ = 168.5 (C=O), 160.2, 130.1 (C≡N), 126.2 (×2), 118.4, 114.2 (×2), 72.9, 55.4, 29.4, 21.0; MS: *m/z* = 219 (M^+), 204, 188, 177, 160, 145, 135, 117, 103, 89, 77, 63, 43.

1d: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (m, 2H, ArH), 7.00 (t, 1H, ArH, *J* = 4.33 Hz), 2.13 (s, 3H, CH_3), 2.10 (s, 3H, CH_3); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 168.4 (C=O), 140.7 (C≡N), 127.3, 126.9, 126.8, 117.6, 69.5, 29.1, 21.1; MS: *m/z* = 195 (M^+), 180, 153, 136, 125, 109, 97, 84, 65, 43.

General Procedure for the Enzymatic Hydrolysis[29,30]

The cyanohydrin acetate **1a–d** (1 mmol) was added to a 10 mM phosphate buffer at pH 7 (10 mL) and 25 °C. The enzyme (200 U) was added and the pH kept constant by the addition of a 0.1 M NaOH solution with an automatic burette. At neutral pH, the acetate was extracted with dichloromethane, dried over $MgSO_4$ and the solvent was removed under vacuum. The *ee* of the remaining acetate was determined by enantioselective GC analysis (see above). The cyanohydrin released by the enzyme decomposed to the corresponding ketone. No other by-products were observed.

An enzymatic hydrolysis reaction on a preparative scale was performed in the same way, using **1a** (2.91 mmol) in a 10 mM phosphate buffer at pH 7 (30 mL) and CRL (600 U). This yielded (*S*)-**1a** as a yellow oil with an *ee* of 67 % as determined by enantioselective GC, $[\alpha]_D^{20}$: +12.2° (*c* 8.65, $CHCl_3$). The already fully characterised (*R*)-**1a** has $[\alpha]_D^{22}$: −15.2° (*c* 0.34, $CHCl_3$).[22]

(*S*)-**1a** (*ee* 67 %) was converted into the corresponding TMS ether of (*S*)-**2a**;[25] $[\alpha]_D^{20}$: −1.89° (*c* 8.75, $CHCl_3$), literature for TMS ether of (*R*)-**2a**[24] $[\alpha]_D^{20}$: +18.5° (*c* 1.25, $CHCl_3$). The sequence of enantiomers of the TMS ethers of **2a** in the enantioselective GC was identical to those described in the literature.[23] The enantiopurity as determined by enantioselective GC of the TMS ether of (*S*)-**2a** was *ee* 66 %.

For **1b–d** the enzymatic hydrolysis was performed with Subtilisin A on a larger scale (2.8 mmol substrate) and the resulting enantioenriched (*R*)-**1b–d** had the following optical rotations and *ee* values (determined by enantioselective GC): (*R*)-**1b** $[\alpha]_D^{20}$: −15.36° (*c* 11.4, $CHCl_3$), *ee* 70 %; (*R*)-**1c** $[\alpha]_D^{20}$: +0.28° (*c* 12.9, $CHCl_3$), *ee* 62 %; and (*R*)-**1d** $[\alpha]_D^{20}$: +3.71° (*c* 14.3, $CHCl_3$), *ee* 8 %.

Acknowledgements

J. H. gratefully acknowledges the National Research School Combination Catalysis (NRSC-Catalysis) for financial support. The authors thank Dr. Lars Veum for suggestions and fruitful discussions.

References

[1] R. J. H. Gregory, *Chem. Rev.* **1999**, *99*, 3649–3682.
[2] M. North, *Tetrahedron: Asymmetry* **2003**, *14*, 147–176.
[3] J.-M. Brunel, I. P. Holmes, *Angew. Chem. Int. Ed.* **2004**, *43*, 2752–2778.
[4] J. Sukumaran, U. Hanefeld, *Chem. Soc. Rev.* **2005**, *34*, 530–542.

[5] Y. N. Belokon, P. Carta, M. North, *Lett. Org. Chem.* **2004**, *1*, 81–83.
[6] T. Purkarthofer, W. Skranc, H. Weber, H. Griengl, M. Wubbolts, G. Scholz, P. Pöchlauer, *Tetrahedron* **2004**, *60*, 735–739.
[7] P. D. de Maria, C. Carboni-Oerlemans, B. Tuin, G. Bargeman, A. van der Meer, R. van Gemert, *J. Mol. Catal. B: Enzym.* **2005**, *37*, 36–46.
[8] E. Henke, J. Pleiss, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2002**, *41*, 3211–3213.
[9] S.-H. Yeo, T. Nihira, Y. Yamada, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 2312–2317.
[10] S. H. Krishna, M. Persson, U. T. Bornscheuer, *Tetrahedron: Asymmetry* **2002**, *13*, 2693–2696.
[11] M. Schmidt, E. Barbayianni, I. Fotakopoulou, M. Höhne, V. Constantinou-Kokotou, U. T. Bornscheuer, G. Kokotos, *J. Org. Chem.* **2005**, *70*, 3737–3740.
[12] E. Henke, U. T. Bornscheuer, R. D. Schmid, J. Pleiss, *ChemBioChem.* **2003**, *4*, 485–493.
[13] H. Ohta, Y. Kimura, Y. Sugano, T. Sugai, *Tetrahedron* **1989**, *45*, 5469–5476.
[14] H. Ohta, Y. Kimura, Y. Sugano, *Tetrahedron Lett.* **1988**, *29*, 6957–6960.
[15] H. Ota, (Central Glass Co., Ltd., Japan), *Japanese Patent* JP 01225497, **1988**.
[16] K. Konigsberger, K. Prasad, O. Repic, *Tetrahedron: Asymmetry* **1999**, *10*, 679–687.
[17] E. Menendez, R. Brieva, F. Rebolledo, V. Gotor, *J. Chem. Soc., Chem. Commun.* **1995**, 989–990.
[18] M. I. Monterde, S. Nazabadioko, F. Rebolledo, R. Brieva, V. Gotor, *Tetrahedron: Asymmetry* **1999**, *10*, 3449–3455.
[19] C. K. Savile, R. J. Kazlauskas, *J. Am. Chem. Soc.* **2005**, *127*, 12228–12229.
[20] L. T. Kanerva, O. Sundholm, *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
[21] L. Veum, M. Kuster, S. Telalovic, U. Hanefeld, T. Maschmeyer, *Eur. J. Org. Chem.* **2002**, 1516–1522.
[22] G. Lin, S. Han, Z. Li, *Tetrahedron* **1999**, *55*, 3531–3540.
[23] Y. Shen, X. Feng, Y. Li, G. Zhang, Y. Jiang, *Eur. J. Org. Chem.* **2004**, 129–137.
[24] H. Deng, M. P. Isler, M. L. Snapper, A. H. Hoveyda, *Angew. Chem. Int. Ed.* **2002**, *41*, 1009–1012.
[25] E. G. J. C. Warmerdam, J. Brussee, C. G. Kruse, A. van der Gen, *Tetrahedron* **1993**, *49*, 1063–1070.
[26] S. S. Kim, D. W. Kim, G. Rajagopal, *Synthesis* **2004**, 213–216.
[27] A. E. Vougioukas, H. B. Kagan, *Tetrahedron Lett.* **1987**, *28*, 5513–5516.
[28] L. Veum, U. Hanefeld, A. Pierre, *Tetrahedron* **2004**, *60*, 10419–10425.
[29] D. Klomp, J. A. Peters, U. Hanefeld, *Tetrahedron: Asymmetry* **2005**, *16*, 3892–3896.
[30] J. Sukumaran, J. van Gool, U. Hanefeld, *Enzyme Microb. Technol.* **2005**, *37*, 254–260.

