Pergamon

Tetrahedron 57 (2001) 2213–2220

TETRAHEDRON

On the selectivity of oxynitrilases towards α-oxygenated aldehydes☆

Paola Bianchi,[a] Gabriella Roda,[a,b] Sergio Riva,[a,*] Bruno Danieli,[b] Antonina Zabelinskaja-Mackova[c] and Herfried Griengl[c]

[a]*Istituto di Biocatalisi e Riconoscimento Molecolare, C.N.R., via Mario Bianco 9, I-20131 Milano, Italy*
[b]*Dipartimento di Chimica Organica ed Industriale, Centro C.N.R. di Studio per le Sostanze Organiche Naturali, Università di Milano, via Venezian 21, I-20133 Milano, Italy*
[c]*Institute of Organic Chemistry, Technical University Graz, Stremayrgasse 16, A-8010 Graz, Austria*

Received 28 September 2000; revised 13 December 2000; accepted 4 January 2001

Abstract—Different α-alkoxy and α,β-di-alkoxy substituted aldehydes have been submitted to the catalytic action of the oxynitrilases from almond (*PaHNL*) or from *Hevea brasiliensis* (*HbHNL*), in order to explore the possibility of using these enzymes for the preparation of complex cyanohydrins. The selectivity of both enzymes towards these compounds was found to be largely dependent on the substitutents, being low with the aldehydes carrying the sterically more demanding phenyl substituent. Contrary to the chemical addition of HCN, which always occurs with a slight preference for the formation of the *anti* diastereoisomers, the enzymatic cyanuration—occurring with a facial preference, *Si* or *Re* according to the biocatalyst used—gave a mixture of cyanohydrins that, depending on the starting enantiomeric aldehyde, can be enriched in the *syn* diastereoisomers.

1. Introduction

Exploitation of oxynitrilases for the stereoselective synthesis of (*R*)- and (*S*)-cyanohydrins from aldehydes and ketones is a well-assessed methodology nowadays.[2] As a part of our general interest in enzymatic catalysis in organic solvents,[3] we have been studying the performances of these biocatalysts towards several different carbonylic substrates and, specifically, focusing attention on the influence of a stereocenter already present in the molecule on the selectivity displayed by these enzymes.[1,4]

In this respect, very recently, we have reported on the influence of simple α- and β-alkoxy substituents on the stereoselectivity displayed by almond oxynitrilase (*PaHNL*) towards a set of aldehydes (i.e. racemic **1**–**3**).[1] As an extension of this previous work, we report here on the results obtained with the racemic α-alkoxy aldehydes **4** (monocyclic analog of **3**) and **5**, and with the α,β-di-alkoxy aldehydes **6**–**8** (enantiomerically pure) and **9** (racemic) (Fig. 1). The diastereoselectivity of HCN addition to these compounds catalyzed by *PaHNL* has been matched with the reaction outcomes obtained with another oxynitrilase with opposite stereospecificity, namely the enzyme from *Hevea brasiliensis* (*HbHNL*)[5] and compared with the data recently reported in the literature for chemical[6] and enzymatic[7] transformations of similar substrates.

2. Results and discussion

2.1. Synthesis of the aldehydes 3–9

Most of the aldehydes have been prepared from the corresponding alcohol using the Swern procedure.[8] Specifically, **4** and **5** were obtained from the commercially available racemic alcohols, while the precursor of **9** was obtained by condensation of glycerol and cyclohexanone.[9]

Aldehyde (*R*)-**6** was obtained from mannitol,[10] and its enantiomer (*S*)-**6** was prepared from a commercially available ascorbic acid derivative.[11]

Aldehyde (2*R*,3*S*)-**7** was prepared from D-threonine according to Scheme 1A, and its enantiomer (2*S*,3*R*)-**7** was similarly obtained from L-threonine.

Finally, the enantiomeric compounds (2*R*,3*S*)-**8** and (2*S*,3*R*)-**8** were prepared via Sharpless hydroxylation of methyl cinnamate in the presence of $(DHQ)_2PHAL$ and $(DHQD)_2PHAL$, respectively (Scheme 1B).[12]

☆ Oxynitrilases in organic synthesis, Part 4.[1]

Keywords: almond oxynitrilase (PaHNL); *Hevea brasiliensis* oxynitrilase (HbHNL); cyanohydrins; aldehyde oxynitrilases.

* Corresponding author. Tel.: +39-02-285-000-00; fax: +39-02-285-000-36; e-mail: rivas@ico.mi.cnr.it

0040–4020/01/$ - see front matter
PII: S0040-4020(01)00054-0

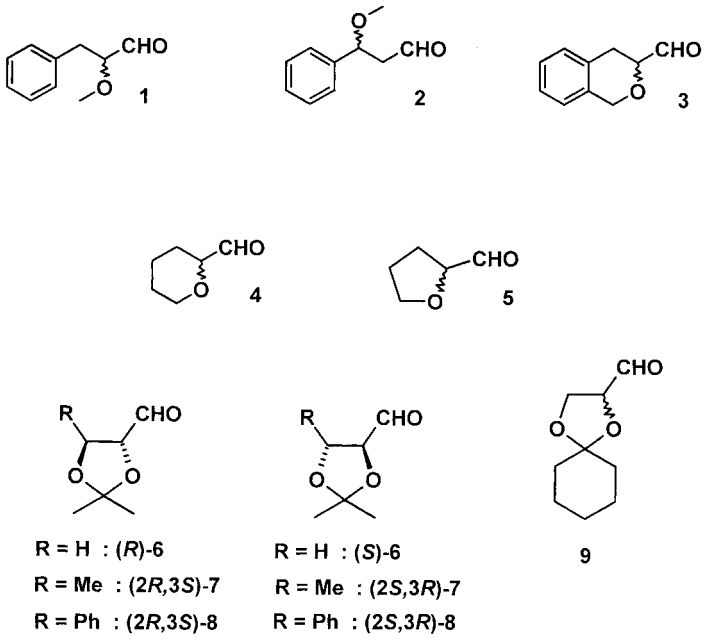
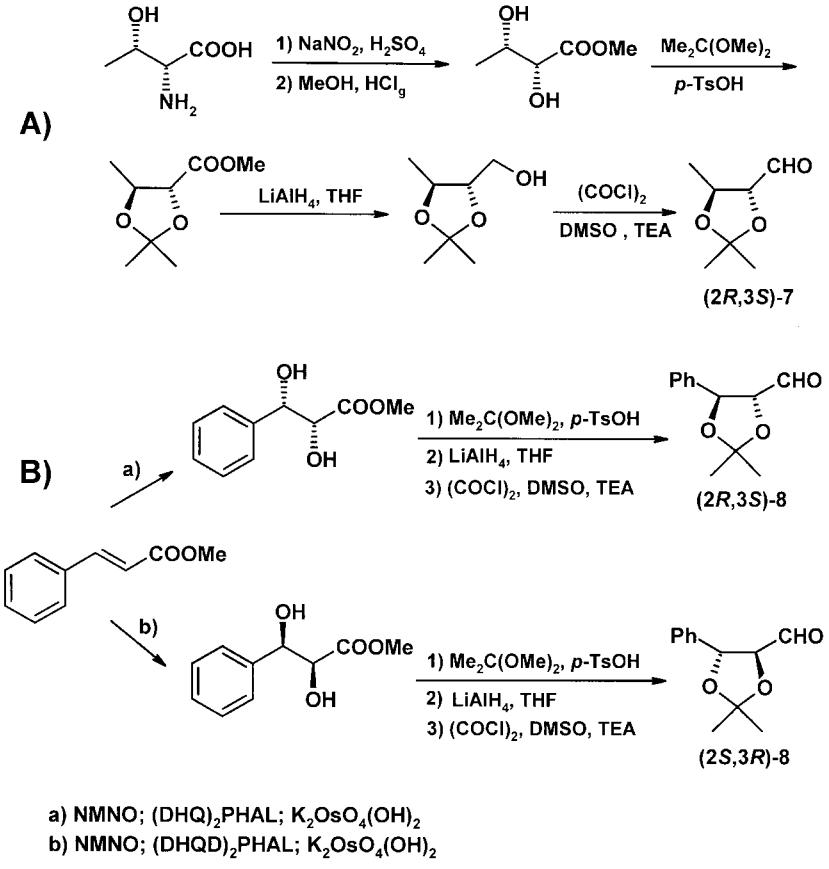


Figure 1.

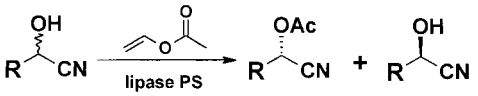
2.2. Stereochemical correlations

The aldehydes **3**–**9** have been chemically transformed into the corresponding cyanohydrins (**Xa–d**). The four diastereoisomers (or the two epimers obtained from the pure enantiomers of the aldehydes **6**–**8**) have been analyzed either by chiral HPLC or, after acylation, by chiral GC, obtaining base-line separated peaks.

The absolute configuration of the stereogenic center of the



Scheme 1.



Scheme 2. Acetylation of racemic cyanohydrins catalyzed by lipase PS.

cyanohydrins was determined by exploiting the well-known selectivity of lipase PS in the acylation of these compounds (Scheme 2).[13] However, it should be noted that, at variance to the usual esterification of (2*S*)-cyanohydrins (R=alkyl or aryl), formally, the (2*R*)-stereoisomers are acylated in the presence of α-oxygenated substituents (R=CHR′OR″), due to the CIP priority rules.

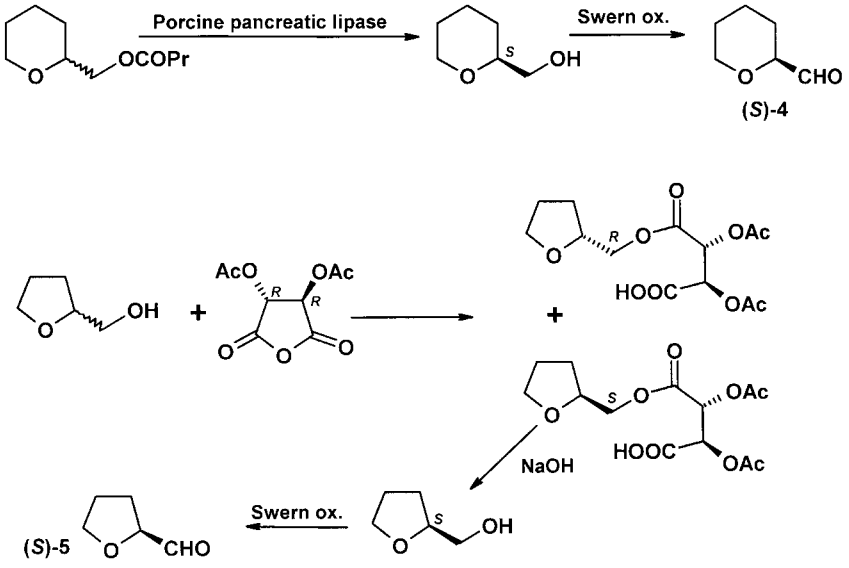
Then, to complete the correlation between the absolute configurations of the products and their own chiral chromatographic peaks, preliminary resolution of the synthetic precursors of the aldehydes **4**[14] and **5**[15] was accomplished (Scheme 3). Additionally, the (*R*)-alcohol, precursor of (*D*)-**9**, was commercially available.

The enantiomerically enriched aldehydes **4**, **5** and **9** were then chemically transformed into the corresponding cyanohydrins, and the diastereoisomeric mixtures, enriched in the products with a defined absolute configuration at C-3, analyzed by chiral chromatographic techniques. The combination of the whole of this information allowed us to evaluate the stereochemical outcome of the enzymatic reactions.

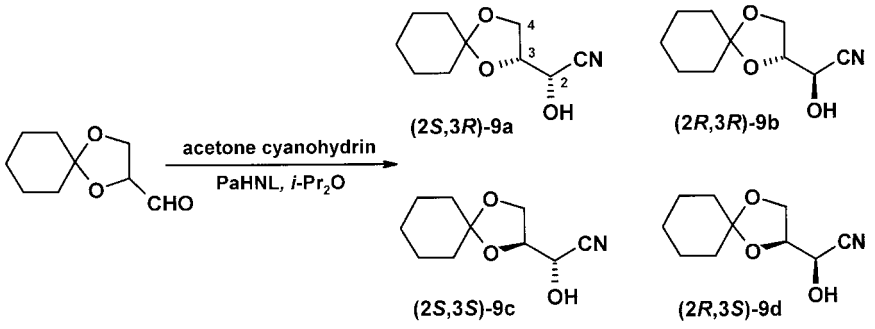
2.3. Almond oxynitrilase-catalyzed synthesis of cyanohydrins

In analogy with our previous investigations, acetone cyanohydrin was used as HCN donor for the *PaHNL*-catalyzed synthesis of cyanohydrins in *iso*-propyl ether, as shown for the aldehyde **9** (Scheme 4).[16] Table 1 reports the results obtained with the aldehydes **4**–**9** ('2' refers to the cyanohydrin stereogenic carbon and '3' refers to its α-carbon, as indicated in compound **9a**). No transformation was observed in the absence of the enzyme.

The data of Table 1 deserve some comments. Concerning substrate reactivity, all the compounds were quantitatively converted to the corresponding cyanohydrins, except (*R*)-**6**. This aldehyde proved to be quite unsuitable for the *PaHNL* active site and, after several attempts, we only observed a 11% conversion at best. Diastereomeric excesses of the products were below 70%, being particularly low with the aldehydes **8** and **9** carrying the more sterically demanding



Scheme 3.



Scheme 4.

Table 1. *PaHNL*-catalyzed transformation of aldehydes **4**–**9** using acetone cyanohydrin as cyanide source

Substrate	Cyanohydrins	% Diastereomeric composition[a]					
		2*S*,3*R* (Xa)	2*R*,3*R* (Xb)	d.e. 3*R*	2*S*,3*S* (Xc)	2*R*,3*S* (Xd)	d.e. 3*S*
4	**4a**–**d**	41.6	13.2	51.8	30.6	14.5	35.7
5	**5a**–**d**	37.9	8.4	63.7	39.7	14.0	47.9
(*R*)-**6**	**6a**,**b**	41.6	58.4	16.8[b]			
(*S*)-**6**	**6c**,**d**				73.8	26.2	47.6
(2*R*,3*S*)-**7**	**7a**,**b**	25.6	74.4	48.8[b]			
(2*S*,3*R*)-**7**	**7c**,**d**				55.1	44.9	10.2
(2*R*,3*S*)-**8**	**8a**,**b**	49.5	50.5	1.0[b]			
(2*S*,3*R*)-**8**	**8c**,**d**				45.3	54.7[a]	9.4
9	**9a**–**d**	23.1	27.3	8.3[b]	29.4	20.2	18.5

[a] In the presence of α-oxygenated substituents, due to the C.I.P. priority rules, formally the (2*S*)-cyanohydrins are the 'natural' products produced by *PaHNL*.
[b] Inversion of the 'natural' expected selectivity.

substituents. Additionally, we observed a peculiar inversion of the natural selectivity towards one of the enantiomers of the dioxolane type substrates **6**–**9**. The 3:1 ratio obtained with (2*R*,3*S*)-**7** in favor of the 'wrong' diastereoisomer is, by far, the highest d.e. value (48.8%) reported up to now, related to the inversion of the natural selectivity of this enzyme.

In order to directly compare our results with some data reported in the literature for the aldehydes (*R*)-**6** and (*S*)-**6**,[7] we repeated these biotransformations using *PaHNL* adsorbed on celite R-630® and HCN as cyanurating agent. Additionally, the reactions were also performed with the (*S*)-specific oxynitrilase from *Hevea brasiliensis* (HbHNL) to compare the catalytic outcomes of two unrelated enzymes. The results are reported in Table 2, together with the diastereomeric composition of the corresponding cyanohydrins obtained by chemical cyanuration of **4**–**9**. (In fact, we observed that the spontaneous addition of the chemical HCN to these compounds was not negligible any

Table 2. Comparison of the performances of *PaHNL* and *HbHNL* with the aldehydes **4**–**9**

Substrate	Method	Product	% Diastereomeric composition[a]					
			2*S*,3*R* (Xa)	2*R*,3*R* (Xb)	d.e. 3*R*	2*S*,3*S* (Xc)	2*R*,3*S* (Xd)	d.e. 3*S*
4	A	**4a**–**d**	47.6	4.6	82.4	42.6	5.2	78.2
	B		5.4	45.9	78.9	3.9	44.9	84.0
	C		22.7	27.6	9.7	27.5	22.2	10.5
5	A	**5a**–**d**	33.7	16.2	35.1	35.6	14.5	42.1
	B		17.1	32.9	31.6	18.9	31.1	24.4
	C		22.4	27.2	9.5	27.3	23.1	8.3
(*R*)-**6**	A	**6a**,**b**	47.1	52.9	5.8[b]			
	B		34.9	65.1	30.2			
	C		42.9	57.1	14.2			
(*S*)-**6**	A	**6c**,**d**				66.4	33.6	32.8
	B					29.8	70.2	40.4
	C					42.2	57.8	15.6
(2*R*,3*S*)-**7**	A	**7a**,**b**	34.2	65.8	31.6[b]			
	B		48.1	51.9	3.8			
	C		47.2	52.8	5.6			
(2*S*,3*R*)-**7**	A	**7c**,**d**				52.6	47.4	5.2
	B					35.1	64.9	29.8
	C					49.1	50.9	1.8
(2*R*,3*S*)-**8**	A	**8a**,**b**	48.2	51.8	3.6[b]			
	B		52.7	47.3	5.4			
	C		49.2	50.8	1.6			
(2*S*,3*R*)-**8**	A	**8c**,**d**				49.3	50.7	1.4[b]
	B					49.9	50.1	0.2
	C					52.4	47.6	4.8
9	A	**9a**–**d**	21.8	28.3	13.0[b]	30.3	19.6	21.4
	B		16.9	33.0	32.3	18.3	31.8	26.9
	C		21.6	28.7	14.1	28.2	21.6	13.3

A: *PaHNL*, HCN; B: *HbHNL*, HCN; C: HCN.
[a] In the presence of α-oxygenated substituents, due to the C.I.P. priority rules, formally the (2*S*)-cyanohydrins are the 'natural' products produced by *PaHNL* and the (2*R*)-cyanohydrins are the 'natural' products produced by *HbHNL*.
[b] Inversion of the 'natural' expected selectivity.

longer after several hours of incubation under these reaction conditions).

As expected, *PaHNL* and *HbHNL* gave complementary reaction outcomes with most of the substrates. However, at variance to *PaHNL*, *HbHNL* did not show any inversion of its natural selectivity with the enantiomers of aldehydes **6**–**9**.

Aldehyde (*R*)-**6** confirmed to be a poor substrate for *PaHNL* and the d.e. (5.8%) was even worse than the one obtained with acetone cyanohydrin (16.8%, Table 1): the conversion was quantitative, but we cannot exclude that it was partly due to spontaneous chemical cyanuration. The results obtained by the action with *HbHNL* on this aldehyde were slightly better in terms of d.e.

The data in Table 2 clearly shows that the selectivity of *HbHNL* towards these compounds was also generally low, particularly with the set of aldehydes **6**–**9**, that never gave d.e. higher than 50%. However, it deserves to be emphasized that, while the chemical addition of HCN to compounds **3**–**9** always occurred with a slight preference for the formation of the *anti* diastereoisomers, the enzymatic cyanuration—occurring with a facial preference, *Si* or *Re* according to the biocatalyst used—gave mixtures of cyanohydrins that, depending on the starting enantiomeric aldehyde, can be enriched in the *syn* diastereoisomers (Table 2).

A possible synthetic application of the cyanuration of these compounds is the so-called Kiliani–Fischer one-carbon homologation of carbohydrates. Using this well established methodology,[17] HCN addition to the starting carbohydrates—followed by acid-catalyzed nitrile hydrolysis, lactonization and reduction to re-establish the aldehydic group—allows the formation of epimeric one-carbon elongated sugars. However, despite the fact that the starting molecules (formally α-hydroxy aldehydes) are enantiomerically pure, poor asymmetric induction has always been observed in this chemical formation of cyanohydrins and therefore new and more selective methodologies would be synthetically quite useful. Unfortunately, the reaction outcomes obtained with the α,β-di-alkoxy aldehydes described in this paper do not support the idea of a more efficient (in term of diastereoselectivity) Kiliani–Fischer sugar homologation using a chemo-enzymatic approach.

Finally, the significant inversion of the natural diastereoselectivity of *PaHNL* with the aldehyde (2*R*,3*S*)-**7** (the same effect observed with (*R*)-**6** and (2*R*,3*S*)-**8**) as well as the similar result previously obtained with 2-naphthyl-acetaldehyde,[1] deserve to be better investigated at a molecular level, as it will be possible as soon as the three-dimensional structure of *PaHNL* will be elucidated.

3. Experimental

3.1. Materials and methods

*Prunus amygdalus*oxynitrilase (*PaHNL*) was isolated from grounded almonds.[18] The oxynitrilase from *Hevea brasiliensis* (HbHNL) was obtained from Roche diagnostics. Lipase PS from *Pseudomonas cepacia* was purchased from Amano. Celite® R-630 was from Fluka. Acetone cyanohydrin and other reagents were from Aldrich. A ~0.6 M HCN solution in methyl *tert*-butyl ether was prepared according to Brussee.[19] HPLC analyses were performed using a Chiralcel OD column (from DIACEL) and a Jasco 880/PU instrument equipped with a Jasco 875 UV/VIS detector (reading was done at 254 nm). GC analyses were performed using a Chrompack capillary column fused silica gel coated with CP-cyclodex B236 M and a Hewlett Packard 5890 series II instrument. FT-IR spectra were recorded using a Jasco 610 instrument equipped with a DTGS detector. ^{1}H NMR spectra were recorded on a Bruker AC-300 at 300 MHz using $CDCl_3$ as a solvent.

3.2. Synthesis of aldehydes

3.2.1. Racemic 4. This compound was prepared from the corresponding alcohol using the Swern procedure[8] and controlling the reaction outcome by TLC (eluent: hexane/AcOEt=7:3). At −60°C, a solution of DMSO (3.7 ml, 51.7 mmol) in CH_2Cl_2 (70 ml) was added dropwise to a 2 M solution of oxalyl chloride (15.5 ml, 31.0 mmol) in CH_2Cl_2. The mixture was stirred for 20 min and then a solution of tetrahydropyran-2-methanol (3 g, 25.8 mmol) in CH_2Cl_2 was added dropwise. The mixture was stirred for 10 min and then Et_3N (18 ml, 129.1 mmol) was slowly added. The reaction mixture was warmed up to room temperature, stirred for 30 min and H_2O was added. When the mixture became clear, it was extracted with CH_2Cl_2, the solvent was evaporated and the product was purified by flash chromatography (eluent: hexane/AcOEt=8:2) followed by bulb-to-bulb distillation (80°C, 20 mmHg) to give the title compound **4** (1.3 g, 44%); [Found: C, 59.8; H, 7.9. $C_5H_8O_2$ requires C, 60.00; H, 8.00%]. FT-IR, ν_{max}($CHCl_3$): 2989, 2867, 1730, 1216, 1074 cm^{-1}. ^{1}H NMR, δ=9.60 (brs, 1H, CHO); 4.05 (td, 1H, J_1=4 Hz, J_2=12 Hz, H-6eq); 3.80 (dd, 1H, J_1=3 Hz, J_2=10 Hz, H-2); 3.52 (dt, 1H, J_1=12 Hz, J_2=4 Hz, H-6ax).

3.2.2. Racemic 5. This compound was prepared similarly from the corresponding alcohol. The product was purified by flash chromatography (eluent: hexane/AcOEt=7:3) followed by bulb-to-bulb distillation (50°C, 20 mmHg) to give the title compound **5** (1.23 g, 42%); [Found: C, 62.9; H, 8.6. $C_6H_{10}O_2$ requires C, 63.16; H, 8.77%]. FT-IR, ν_{max}($CHCl_3$): 2944, 2861, 1741, 1217, 1094 cm^{-1}. ^{1}H NMR, δ=9.65 (brs, 1H, CHO), 4.26 (dt, 1H, J_1=2 Hz, J_2=8 Hz, H-5a), 3.90–3.70 (m, 2H, H-2 and H-5b).

3.2.3. 2,3-*O*-Isopropylidene-D-glyceraldehyde ((*R*)-6). This compound was prepared from D-mannitol according to a published procedure.[10] (A) Under inert atmosphere, the catalyst stannous chloride (15 mg) was added to a solution of D-mannitol (10 g, 55 mmol) in 2,2-dimethoxypropane (16 ml) and dimethoxyethane (24 ml). The mixture was refluxed until the starting material disappeared (TLC: $CHCl_3$/MeOH=10:0.2), then it was cooled to room temperature and pyridine (20 μl) was added. The solvent was evaporated and the white solid was dissolved in CH_2Cl_2 (100 ml) by heating at the boiling point. The

mixture was filtered, CH_2Cl_2 was evaporated and the solid residue was crystallized from diisobutyl ether to give pure mannitol diacetonide. Yield 7.15 g (50%). (b) A saturated solution of $NaHCO_3$ (1 ml) was added to a solution of the previously obtained mannitol diacetonide in CH_2Cl_2 (20 ml). The mixture was cooled at 0°C and sodium metaperiodate (3.29 g) was slowly added (30 min), keeping the temperature under 35°C (TLC: $CHCl_3$/MeOH=10:0.2). After 4 h, magnesium sulphate (1 g) was added and after 10 min, the mixture was filtered. The solvent was evaporated and the residue was purified by bulb-to-bulb distillation (70°C, 20 mmHg). FT-IR, ν_{max}($CHCl_3$): 2945, 2870, 1723, 1423, 1215 cm^{-1}. ^1H NMR: δ=9.70 (d, 1H, J=3 Hz, CHO); 4.40 (dt, 1H, J_1=3 Hz, J_2=7.8 Hz, H-2); 4.25–4.00 (2dd, each 1H, J_1=8 Hz, J_2=10 Hz, H-3a and H-3b); 1.50 (s, 3H, CH_3); 1.40 (s, 3H, CH_3).

3.2.4. 2,3-*O*-Isopropylidene-L-glyceraldehyde ((*S*)-6). This compound was prepared from 5,6-*O*-isopropylidene-L-gulono-1,4-δ-lactone according to a published procedure.[11] At 0°C, a 3 M NaOH solution was added to a sodium metaperiodate solution (9.8 g in 23 ml of H_2O) keeping the temperature under 5°C until pH 5.5 was reached. The mixture was warmed up to room temperature and 5,6-*O*-isopropylidene-L-gulono-1,4-δ-lactone (5 g, 22.9 mmol) was added. During the reaction, the pH was kept at 5.5 by adding 15% w/v Na_2CO_3. When the reaction was finished (TLC: hexane/AcOEt=7:3), the mixture was saturated with NaCl (12 g) and filtered. The water solution was extracted with CH_2Cl_2. The extracts were dried with Na_2SO_4 and the solvent was evaporated to give the aldehyde that was purified by bulb-to-bulb distillation (70°C, 20 mmHg). ^1H NMR, δ=9.70 (d, 1H, J=3 Hz, CHO); 4.40 (dt, 1H, J_1=3 Hz, J_2=8 Hz, H-2); 4.25-4.00 (2dd, each 1H, J_1=8 Hz, J_2=10 Hz, H-3a and H-3b); 1.50 (s, 3H, CH_3); 1.40 (s, 3H, CH_3).

3.2.5. 4-Deoxy-2,3-*O*-isopropylidene-L-threose ((2*R*,3*S*)-7). This compound was prepared from D-threonine. (A) At −5°C, a solution of $NaNO_2$ (3.13 g, 0.045 mol) in H_2O (6.6 ml) and a solution of 95–97% H_2SO_4 (1.28 ml, 0.023 mol) in H_2O (3.3 ml) were added, slowly and simultaneously, to a solution of D-threonine (5 g, 0.042 mol) in H_2O (50 ml) keeping the temperature under 5°C. The reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue was dissolved in 50 ml of EtOH. The solid residue was filtered through celite and the solvent was evaporated. The residue was esterified with MeOH (40 ml) saturated with gaseous HCl. (B) 2,2-Dimethoxypropane (5.04 ml, 0.042 mol) and catalytic *p*-toluenesulfonic acid were added to the methyl ester dissolved in acetone (17 ml) and the reaction mixture was allowed to stand for 4 h at room temperature (TLC: hexane/AcOEt=7:3). The product was purified by flash chromatography (eluent: hexane/AcOEt=7:3). Total yield 3.9 g (53%). (C) Under inert atmosphere at 0°C, a solution of the acetonide ester (3.9 g, 22.41 mmol) in THF (60 ml) was carefully dropped into a 1.0 M solution of $LiAlH_4$ in THF (23 ml). After 10 min, the mixture was warmed up to room temperature. (TLC: hexane/AcOEt=7:3). The excess of $LiAlH_4$ was destroyed by adding AcOEt, then 1.5 ml of H_2O was added and the salts were filtered through celite. The solution was dried (Na_2SO_4) and the solvent was evaporated to give the alcohol that was purified by bulb-to-bulb distillation (115°C, 20 mmHg). Yield 3 g (92%). (D) The alcohol was oxidized using the Swern protocol.[8] The product was purified by flash chromatography (eluent: hexane/AcOEt=75:25) and followed by bulb-to-bulb distillation (100°C, 20 mmHg) to give the title compound ((2*R*,3*S*)-**7**) (1.35 g, 46%); [Found: C, 58.1; H, 8.2. $C_7H_{12}O_3$ requires C, 58.33; H, 8.33%]; FT-IR: ν_{max}($CHCl_3$): 2990, 2895, 1721, 1454, 1382, 1252, 1098 cm^{-1}. ^1H NMR, δ=9.65 (d, 1H, J=3 Hz, CHO); 4.11 (dq, 1H, J_1=8 Hz, J_2=6 Hz, H-3); 3.81 (dd, 1H, J_1=8 Hz, J_2=3 Hz, H-2); 1.49 (s, 3H, CH_3–C); 1.41 (s, 3H, CH_3–C); 1.39 (d, 3H, J=6 Hz, CH_3–CH).

3.2.6. 4-Deoxy-2,3-*O*-isopropylidene-D-threose ((2*S*,3*R*)-7). This compound was prepared similarly from L-threonine.

3.2.7. (3*S*)-3-Phenyl-2,3-*O*-isopropylidene-D-glyceraldehyde ((2*R*,3*S*)-8). This compound was prepared from methyl cinnamate. (A) To *N*-methylmorpholino-*N*-oxide (NMNO) at 60% (40 ml), a solution of cinnamate (5 g, 30.86 mmol) in *tert*-butanol (200 ml) was added, followed by the addition of $(DHQ)_2$(PHAL) (62 mg) and $K_2OsO_4(OH)_4$ (127 mg); (TLC, hexane/AcOEt=7:3). After 28 h, a solution of sodium sulphite in 80 ml of water was added, the reaction mixture was stirred for 1 h and the water was evaporated. The residue was extracted with AcOEt and washed with 1N HCl and then with brine. The organic phase was evaporated and the solid recrystallized from AcOEt. Yield 4.23 g (70%). (B) 2,2-Dimethoxypropane (5.3 ml, 0.44 mol) and catalytic *p*-toluenesulfonic acid were added to the residue in CH_2Cl_2 (13 ml; TLC: hexane/AcOEt=7:3). When the reaction was finished, the solvent was evaporated and the residue reduced to the alcohol (1 M $LiAlH_4$ in THF, yield 3.3 g, 73%), then oxidized to the aldehyde using the Swern protocol[8] and finally purified by flash chromatography (eluent: $CHCl_3$/MeOH=99:1) followed by bulb-to-bulb distillation (125°C, 5 mmHg) to give the title compound ((2*R*,3*S*)-**8**) (1.8 g, 55%); [Found: C, 69.6; H, 6.6. $C_{12}H_{14}O_3$ requires C, 69.90; H, 6.80%]; FT-IR, ν_{max}($CHCl_3$): 2996, 2942, 2902, 1718, 1461, 1385, 1238 cm^{-1}. ^1H NMR, δ=9.82 (d, 1H, J=2 Hz, CHO); 7.30–7.50 (m, 5H, Ph); 5.09 (d, 1H, J=8 Hz, H-3); 4.21 (dd, 1H, J_1=8 Hz, J_2=2 Hz, H-2); 1.45 (s, 3H, CH_3); 1.42 (s, 3H, CH_3).

3.2.8. (3*R*)-3-Phenyl-2,3-*O*-isopropylidene-L-glyceraldehyde (2*S*,3*R*)-8. This compound was prepared similarly using the enantiomeric catalyst $(DHQD)_2$PHAL.

3.2.9. Racemic 2,3-*O*-cyclohexylidene-D,L-glyceraldehyde 9. This compound was prepared by condensation of glycerol and cyclohexanone (flash chromatography, eluent: hexane/AcOEt=7:3),[9] followed by oxidation according to the Swern procedure,[8] and final purification by flash chromatography (eluent: $CHCl_3$/MeOH=99:1, yield of the Swern oxidation 2 g, 67%), followed by bulb-to-bulb distillation (100°C, 5 mmHg) to give the title compound **9**; [Found: C, 63.2; H, 8.1. $C_9H_{14}O_3$ requires C, 69.90; H, 6.80%]. FT-IR, ν_{max}($CHCl_3$): 2943, 2859, 1739, 1217, 1098 cm^{-1}. ^1H NMR, δ=9.71 (d, 1H, J=2 Hz, CHO); 4.0–3.6 (m, 3H, H-2 and CH_2-3); 1.80–1.30 (m, 10H, cyclohexyl moiety).

3.3. General procedure for the chemical synthesis of cyanohydrins (Method C of Table 2)

To a solution of 20 mg of aldehyde in 1 ml of 80% v/v AcOH, NaCN (3 equiv.) dissolved in 1 ml of water was added dropwise at 0°C. When the reaction was over, water was added and the mixture was extracted with ethyl ether. The organic phase was washed with $NaHCO_3$, dried with Na_2SO_4 and evaporated. The cyanohydrins mixtures (two epimers or four diastereoisomers) were purified by flash chromatography on silica using a mixture of hexane/AcOEt (7:3 for **4a–d**, **5a–d**, and **6a–d**; 8:2 for **7a–d**, **8a–d**, and **9a–d**) as the eluent.

3.3.1. Compounds 4a–d. Found: C, 56.4; H, 6.9. $C_6H_9O_2N$ requires C, 56.69; H, 7.09%. GC-analysis (as acetates), T_i=50°C; t_i=5 min; rate=3°C/min; T_f=200°C; ret. times (min): **4a**, 44.160; **4b**, 43.272; **4c**, 43.108; **4d**, 44.410. FT-IR, ν_{max}($CHCl_3$): 3650–3100 (br), 2984, 2875, 2250, 1219, 1075 cm^{-1}. ^1H NMR (for carbon number see Scheme 4): δ=4.34 and 4.29 (2d, 0.5H each, J=6 Hz, CH(CN)OH); 4.10 (m, 1H, H-6eq); 3.50 (m, 2H, H-2 and H-6ax).

3.3.2. Compounds 5a–d. Found: C, 59.4; H, 7.6. $C_7H_{11}O_2N$ requires C, 59.57; H, 7.80%. GC-analysis (as acetates), T_i=100°C; t_i=5 min; rate=0.3°C/min; T_f=200°C; ret. times (min): **5a**, 56.115; **5b**, 49.025; **5c**, 46.377; **5d**, 57.629. FT-IR, ν_{max}($CHCl_3$): 3640–3120 (br), 2945, 2862, 2251, 1447, 1222, 1086 cm^{-1}. ^1H NMR, δ=4.43 and 4.41 (2d, 0.5 H each, J=4 Hz, CH(CN)OH); 4.18 and 3.95 (m, 3H, H-2 and CH_2-5).

3.3.3. Compounds 6a,b. Found: C, 53.6; H, 6.9. $C_7H_{11}O_3N$ requires C, 53.50; H, 7.01%. GC-analysis (as acetates), T_i=50°C; t_i=1 min; rate=5°C/min; T_f=200°C; ret. times (min): **6a**, 25.521; **6b**, 24.655. FT-IR, ν_{max}($CHCl_3$): 3600–3100 (br), 2943, 2896, 2251, 1423, 1215 cm^{-1}. ^1H NMR, as acetates: diastereoisomer 2*R*,3*R*, δ=5.44 (d, 1H, J=6 Hz, H-2); 4.35 (m, 1H, H-3); 4.18 (dd, 1H, J_1=10 Hz, J_2=7 Hz, H-4a); 4.05 (dd, 1H, J_1=10 Hz, J_2=5 Hz, H-4b); 2.16 (s, 3H, CH_3–CO); 1.5 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); diastereoisomer 2*S*,3*R*, δ=5.30 (d, 1H, J=6 Hz, H-2); 4.39 (m, 1H, H-3); 4.15 (dd, 1H, J_1=9 Hz, J_2=6 Hz, H-4a); 3.92 (dd, 1H, J_1=9 Hz, J_2=4 Hz, H-4b); 2.17 (s, 3H, CH_3–CO); 1.5 (s, 3H, CH_3); 1.4 (s, 3H, CH_3). Compounds **6c**,**d**. GC-analysis (as acetates), T_i=50°C; t_i=1 min; rate=5°C/min; T_f=200°C; ret. times (min): **6c**, 24.392; **6d**, 25.520. ^1H NMR, as acetates: diastereoisomer 2*S*,3*S*, δ=5.44 (d, 1H, J=6 Hz, H-2); 4.35 (m, 1H, H-3); 4.18 (dd, 1H, J_1=10 Hz, J_2=7 Hz, H-4a); 4.05 (dd, 1H, J_1=10 Hz, J_2=5 Hz, H-4b); 2.16 (s, 3H, CH_3–CO); 1.5 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); diastereoisomer 2*R*,3*S*, δ=5.30 (d, 1H, J=6 Hz, H-2); 4.39 (m, 1H, H-3); 4.15 (dd, 1H, J_1=9 Hz, J_2=6 Hz, H-4a); 3.92 (dd, 1H, J_1=9 Hz, J_2=4 Hz, H-4b); 2.17 (s, 3H, CH_3–CO); 1.5 (s, 3H, CH_3); 1.4 (s, 3H, CH_3).

3.3.4. Compounds 7a,b. Found: C, 56.0; H, 7.4. $C_8H_{13}O_3N$ requires C, 56.14; H, 7.60%. GC-analysis (as acetates), T_i=50°C; t_i=1 min; rate=5°C/min; T_f=200°C; ret. times (min): **7a**, 24.031; **7b**, 24.780. FT-IR: ν_{max}($CHCl_3$) 3630–3076 (br), 2992, 2904, 2250, 1454, 1382, 1252, 1098 cm^{-1}. ^1H NMR: δ=4.52 (d, 1H, J=5 Hz, H-2); 4.14 (m, 1H, H-3); 3.75 (m, 1H, H-4); 1.45 and 1.43 (2s, 3H, CH_3); 1.38 and 1.37 (2d, 1.5H each, J=6 Hz, CH_3–CH). Compounds **7c**,**d**. GC-analyses (as acetates), T_i=50°C; t_i=1 min; rate=5°C/min; T_f=200°C; ret. times (min): **7c**, 23.705; **7d**, 24.470. ^1H NMR: δ=4.52 (d, 1H, J=5 Hz, H-2); 4.14 (m, 1H, H-3); 3.75 (m, 1H, H-4); 1.45 and 1.43 (2s, 3H, CH_3); 1.38 and 1.37 (2d, 1.5H each, J=6 Hz, CH_3–CH).

3.3.5. Compounds 8a,b. Found: C, 66.7; H, 6.4. $C_{13}H_{15}O_3N$ requires C, 66.95; H, 6.44%. HPLC analysis, eluent: hexane/^{i}PrOH=96:4; flow rate=0.5 ml/min; ret. time (min): **8a**, 57.31; **8b**, 33.58. FT-IR, ν_{max}($CHCl_3$): 3620–3120 (br), 2993, 2944, 2907, 2252, 1461, 1385, 1238 cm^{-1}. ^1H NMR: δ=7.30–7.45 (m, 5H, Ph); 4.98 and 4.86 (2d, 0.5 H each, J=8 Hz, H-4); 4.51 and 4.48 (d, 0.5 H each, J=3 Hz, H-2); 4.05 and 4.02 (2dd, 0.5 H each, J_1=9 Hz, J_2=4 Hz, H-3); 1.66 and 1.64 (2s, 1.5 H each, CH_3–C); 1.52 and 1.50 (2s, 1.5 H each, CH_3–C). Compounds **8c**,**d**. HPLC analysis, eluent: hexane/*i*PrOH=96:4; flow rate=0.5 ml/min; ret. time (min): **8c**, 31.31; **8d**, 28.46. ^1H NMR: δ=7.30–7.45 (m, 5H, Ph); 4.98 and 4.86 (2d, 0.5 H each, J=8 Hz, H-4); 4.51 and 4.48 (d, 0.5 H each, J=3 Hz, H-2); 4.05 and 4.02 (2dd, 0.5 H each, J_1=9 Hz, J_2=4 Hz, H-3); 1.66 and 1.64 (2s, 1.5 H each, CH_3–C); 1.52 and 1.50 (2s, 1.5 H each, CH_3–C).

3.3.6. Compounds 9a–d. Found: C, 66.4; H, 8.2. $C_{10}H_{15}O_2N$ requires C, 66.30; H, 8.29%. GC-analysis (as butanoates), T_i=100°C; t_i=5 min; rate=1°C/min; T_f=200°C; ret. times (min): **9a**, 93.257; **9b**, 89.885; **9c**, 89.377; **9d**, 92.840. FT-IR: ν_{max}($CHCl_3$): 3600–3080 (br), 2940, 2860, 2252, 1217, 1098 cm^{-1}. ^1H NMR: δ=4.44 and 4.42 (2d, 0.5 H each, J=4 Hz, H-2); 4.31 (m, 1H, H-3); 4.14 (m, 1H, H-4a); 3.99 (m, 1H, H-4b).

3.4. General procedures for the enzymatic synthesis of the cyanohydrins

3.4.1. Using acetone cyanohydrin (Table 1). To a solution of 250 mg of aldehyde in 10 ml of isopropyl ether containing 1.3 equiv. of acetone cyanohydrin, *PaHNL* (~1500 units) dissolved in 500 μl of 0.1 M citrate buffer pH 5.5, was added and the biphasic system shaken at room temperature for 5 days. At the end of the reaction, the two phases were separated. The aqueous phase was extracted with isopropyl ether, the organic phases were dried with Na_2SO_4 and evaporated. The cyanohydrins were purified by flash chromatography.

3.4.2. Using HCN (Methods A and B of Table 2). *Method A. PaHNL* (~400 units), dissolved in 100 μl of 0.1 M citrate buffer pH 5.5, was dropped homogeneously on celite (300 mg). The catalyst was added to isopropyl ether containing 250 mg of aldehyde and 2 equiv. of HCN, and the reaction was shaken at room temperature at 150 rpm for 3 days. At the end of the reaction, the mixture was filtered, celite was washed with isopropyl ether and the organic phases were evaporated. The cyanohydrins were purified by flash chromatography.

Method B. HbHNL (~6360 units in 1.2 ml of citrate buffer pH 4.5) was added to a solution of 250 mg of aldehyde in 1.5 ml of isopropyl ether. Neat HCN (5 equiv.) was added at 0°C, and the reaction was stirred at 15°C until the complete

conversion of the substrate was reached. Celite was added to absorb the enzyme and the aqueous phase; the organic phase was dried with Na_2SO_4 and evaporated. The cyanohydrins were purified by flash chromatography.

3.5. General procedure for the acylation with lipase PS

Approximately 30 mg/ml of cyanohydrin were dissolved in methyl *tert*-butylether containing 10% v/v vinyl acetate. Lipase PS immobilised on celite[13] (20 mg/ml) was added, and the suspension was shaken at room temperature until about 50% of conversion was reached. The enzyme was filtered, the solvent was evaporated and the products were purified by flash chromatography.

3.6. Stereochemical correlations

3.6.1. (*S*)-4. This compound was prepared by oxidation of (*S*)-tetrahydropyran-2-methanol according to a published procedure.[14] This alcohol was obtained via kinetic resolution of the racemic butanoyl ester catalyzed by porcine pancreatic lipase (Scheme 3). The enzyme was added to a solution of the racemic ester (2 ml) in phosphate buffer pH 7.2. The mixture was shaken at room temperature (TLC: hexane/AcOEt=8:2), and after 6 h, extracted with AcOEt. The organic phase was dried with Na_2SO_4 and evaporated. The product was purified by flash chromatography (eluent: hexane/AcOEt=9:1, then 8:2, then 1:1).

3.6.2. (*S*)-5. This compound was prepared by oxidation of (*S*)-tetrahydrofuran-2-methanol. This racemic alcohol was resolved into its enantiomers by crystallizing the half esters formed with *O*,*O*′-diacetyl-(2*R*,3*R*)-tartaric acid according to a published procedure (Scheme 3).[15] To a stirred mixture of racemic alcohol (2.37 g, 23.2 mmol) and pyridine (185 mg, 2.3 mmol), (2*R*,3*R*)-diacetyltartaric anhydride (5 g, 23.1 mmol) was added. Stirring was continued for 20 min at room temperature, then 0.5 ml of ethyl acetate was added and the temperature was raised to 80–85°C. After 1.5 h heating, 3 ml of ethyl acetate was added, and the mixture was allowed to cool to room temperature, then kept for 10 h at 5°C. The crystals were filtered, washed with ethyl acetate and dried. $[\alpha]^{25}_{D}=-13.3$ (*c* 1.2, $CHCl_3$; lit.: − 13.6). The solid was slowly added to 25 ml of a stirred solution of NaOH 1 M and allowed to stand for 18 h at room temperature (TLC: hexane/AcOEt=7:3). The aqueous solution was extracted with CH_2Cl_2; Na_2SO_4 (2 g) was dissolved in the aqueous phase, and this was extracted again with CH_2Cl_2. The organic phases were dried with Na_2SO_4 and evaporated.

3.6.3. (*R*)-9. This compound was obtained by oxidation of the corresponding commercially available alcohol.

Acknowledgements

Grants obtained within the framework of the Italian–Austrian treaty on scientific and technological cooperation are gratefully acknowledged. Work in Graz was also financed from the special research program 'Biocatalysis' of the Austrian Research Fund.

References

1. For Part 3, see: Roda, G.; Riva, S.; Danieli, B. *Tetrahedron: Asymmetry* **1999**, *10*, 3939–3949.
2. (a) Griengl, H.; Schwab, H.; Fechter, M. *TIBTECH* **2000**, *18*, 252–256. (b) Schmidt, M.; Griengl, H. *Top. Curr. Chem.* **1999**, *200*, 194–225. (c) Effenberger, F. *Chimia* **1999**, *53*, 3–10. (d) Griengl, H.; Hickel, A.; Johnson, D. V.; Kratky, C.; Schmidt, M.; Schwab, H. *J. Chem. Soc., Chem. Commun.* **1997**, 1933–1940.
3. (a) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 4000–4028. (b) Riva, S. In *Enzymatic reactions in organic media*; Koskinen, A. P. G., Klibanov, A. M., Eds.; Blackie A and P: London, 1995; pp 140–169. (c) Carrea, G.; Ottolina, S.; Riva, S. *Trends Biotechnol.* **1995**, *13*, 63–70. (d) Danieli, B.; Riva, S. *Pure Appl. Chem.* **1994**, *66*, 2215–2218.
4. (a) Danieli, B.; Frattini, S.; Roda, G.; Carrea, G.; Riva, S. *J. Mol. Catal. B: Enzymatic* **1998**, *5*, 223–228. (b) Danieli, B.; Barra, C.; Carrea, S.; Riva, S. *Tetrahedron: Asymmetry* **1996**, *7*, 1675–1682.
5. Schmidt, M.; Herve, S.; Klempier, N.; Griengl, H. *Tetrahedron* **1996**, *52*, 7833–7840 (and references therein).
6. Ward, D. E.; Hrapchak, M. J.; Sales, M. *Org. Lett.* **2000**, *2*, 57–60.
7. Roos, J.; Effenberger, F. *Tetrahedron: Asymmetry* **1999**, *10*, 2817–2828.
8. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165–185.
9. Renoll, M.; Newman, M. S. *Org. Synth.* **1955**, *3*, 502–504.
10. Schmid, R.; Bryant, J. D. *Org. Synth.* **1993**, *72*, 6–12.
11. Hubschwerlen, C.; Specklin, J.-L.; Higelin, J. *Org. Synth.* **1993**, *72*, 1–5.
12. Wang, Z.-M.; Kolb, H. C.; Sharpless, K. B. *J. Org. Chem.* **1994**, *59*, 5104–5105.
13. Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *J. Org. Chem.* **1992**, *57*, 5643–5649.
14. Kwatelay Quartey, E. G.; Hustad, J. A.; Faber, K.; Anthonsen, T. *Enzyme Microb. Technol.* **1996**, *19*, 361–366.
15. Mravik, A.; Böcskei, Z.; Keszei, S.; Elekes, F.; Fogassy, E. *Tetrahedron: Asymmetry* **1996**, *7*, 1477–1484.
16. Ognyanov, F.; Datcheva, V. K.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 6992–6996.
17. (a) Kiliani, H. *Ber. Dtsch. Chem. Ges.* **1885**, *18*, 3066–3072. (b) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1889**, *22*, 2204–2205.
18. Chiari, M.; Gelain, A.; Riva, S.; Tura, D. *Electrophoresis* **1997**, *18*, 2050–2054.
19. (a) Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. In *Preparative biotransformation: Whole cells and isolated enzymes*; Roberts, S. M., Ed.; Wiley: New York, 1995 (Module 4.5.1). (b) Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. *Synth. Commun.* **1991**, *21*, 1387–1395.