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Almond oxynitrilase-catalyzed transformation of aldehydes is strongly influenced by naphthyl and alkoxy substituents

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Abstract

Different α- and β-substituted aldehydes have been submitted to the catalytic action of almond oxynitrilase (PaHNL), in order to explore the influence of a stereocenter already present in the substrate on the selectivity of this enzyme.[1] The results indicate that naphthyl and alkoxy substituents in the α- and also in the β-position to the aldehyde group significantly influence the stereochemical outcome of the PaHNL-catalyzed transformation.

1. Introduction

Exploitation of enzymes that are able to catalyze carbon–carbon bond forming reactions is presently one of the key issues of biocatalysis.[2] Specifically, oxynitrilases — enzymes that catalyze the stereoselective synthesis of (*R*)- or (*S*)-cyanohydrins from aldehydes and ketones — have been isolated from various sources and their catalytic properties studied and widely exploited with natural and non-natural substrates.[3]

As a part of our research on the performances of enzymes in organic solvents,[4] we have included oxynitrilases[1,5] and, specifically, we have been studying the influence of a stereocenter already present in the molecule on the selectivity displayed by these biocatalysts. Several aldehydes carrying a stereocenter with alkyl and/or aryl substituents either in the α- or in the β-position to the carbonyl moiety have been considered so far, using the oxynitrilase isolated from almond (PaHNL). We have found that only when the stereocenter is adjacent to the aldehyde group (as in **4**, **11** and **12**) is a strong influence on the selectivity of this enzyme observed, resulting in the formation of the four possible cyanohydrins although

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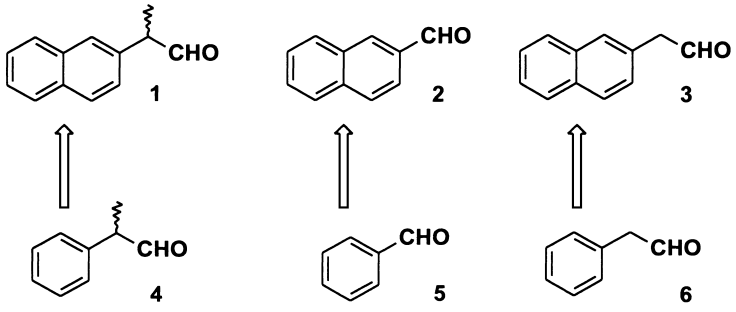
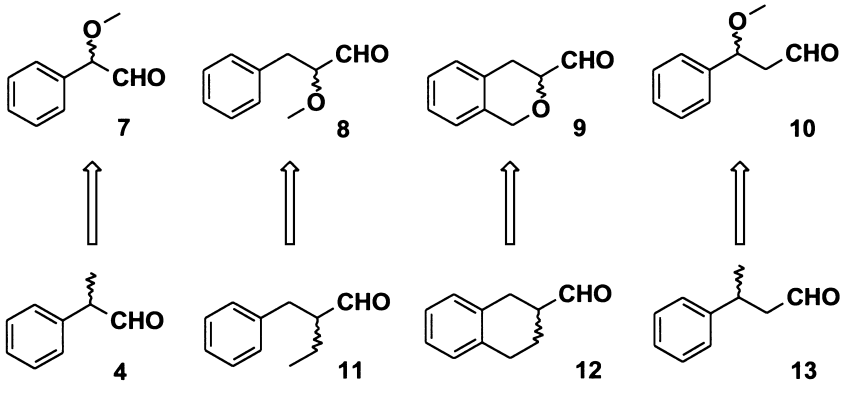
in different ratios; on the other hand, the cyanohydrins obtained from β-substituted aldehydes (as in **13**) had the expected *2R* configuration.[1,5]

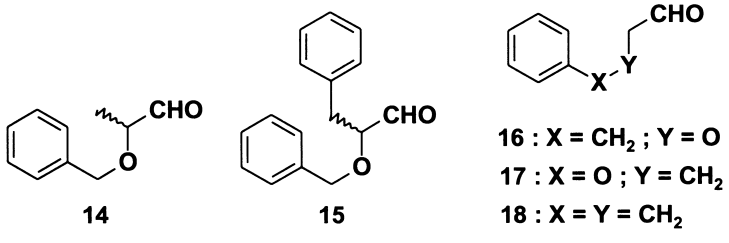
As an extension of the previous work, this paper reports on the results obtained with the naphthyl aldehydes **1**–**3**, analogs of aldehydes **4**–**6** (Scheme 1). The oxygenated aldehydes **7**–**10** and the related compounds **14**–**17** have been considered next, and their behaviors compared to those of their non-oxygenated analogs **4**, **11**–**13**, and **18** (Scheme 2).



Scheme 1.



Scheme 2.

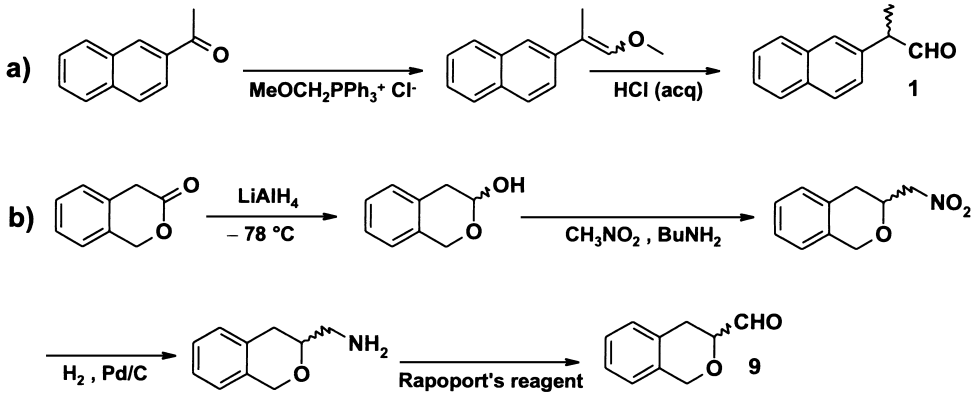


2. Results and discussion

*2.1. Synthesis of the aldehydes **1**, **3**, **7–10**, **14**, **15** and **17**, **18***

Most of these aldehydes have been prepared by oxidation of the corresponding alcohols either using the Swern procedure[6] or the NaOCl/TEMPO system.[7] In turn, the alcohols have been prepared from

commercially available starting materials following straightforward literature procedures. As shown in Scheme 3a, racemic 2-(2-naphthyl)propanal **1** has been prepared from naphthylmethyl ketone via a Wittig condensation followed by acid hydrolysis. In turn, the racemic aldehyde **9** has been synthesized from 3-isochromanone in 4 steps (Scheme 3b).

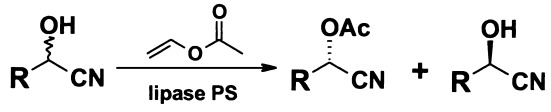


Scheme 3. Synthesis of the aldehydes **1** and **9**

2.2. Stereochemical correlations

The aldehydes **1**–**3**, **7**–**10** and **14**–**18** have been chemically transformed into the corresponding cyanohydrins. The four diastereoisomers (or the two enantiomers obtained from **2**, **3** and **16**–**18**) have base-line separation either by chiral HPLC or, after acetylation, by chiral GC.

As shown in Scheme 4, the absolute configuration of the stereogenic center of the cyanohydrins was determined by exploiting the well-known selectivity of lipase PS in the acetylation of these compounds.[8] However, at variance with the usual esterification of (2*S*)-cyanohydrins (R=alkyl or aryl), formally the (2*R*)-stereoisomers are acetylated in the presence of α-oxygenated substituents (R=CHR′OR′′), due to the CIP priority rules.



Scheme 4. Acetylation of racemic cyanohydrins catalyzed by lipase PS

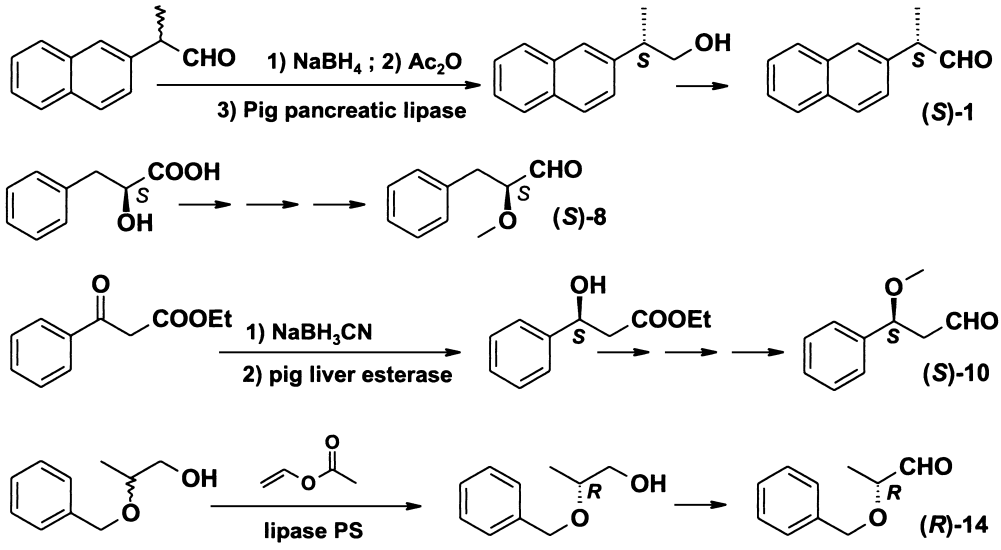
Then, to complete the correlation between the absolute configurations of the products and their own chiral chromatographic peaks, preliminary resolution of the synthetic precursors of the aldehydes **1**,[9] **8**, **10**,[10] and **14**[11] was accomplished, as shown in Scheme 5.

This information allowed us to evaluate the stereochemical outcome of the enzymatic reactions.

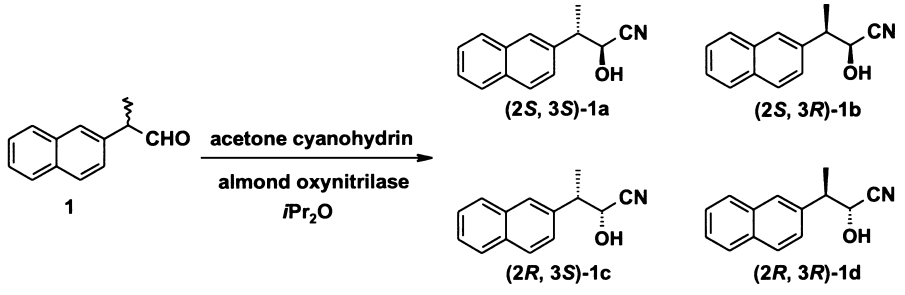
2.3. Almond oxynitrilase-catalyzed synthesis of cyanohydrins

Acetone cyanohydrin was used as HCN donor for the PaHNL-catalyzed synthesis of cyanohydrins in isopropyl ether, as shown for the aldehyde **1** (Scheme 6).[12] No transformation was observed in the absence of the enzyme.

Table 1 reports the results obtained with the naphthyl aldehyde **1** in comparison with the data of the corresponding phenyl analog **4**.



Scheme 5.



Scheme 6.

Table 1
PaHNL-catalyzed transformation of aldehydes **1** and **4**

Substrate	Product	% Diastereomeric composition			
		2*S*, 3*S* (Xa)	2*R*, 3*S* (Xb)	2*S*, 3*R* (Xc)	2*R*, 3*R* (Xd)
1	1a-d	30.4	24.0	20.0	25.6
4	4a-d	3.0	51.8	27.6	17.6

2-(2-Naphthyl)propanal **1** was prepared to evaluate the effect of a hydrophobic large substituent on PaHNL-selectivity. As shown in Table 1, almost no enantiomeric and diastereomeric discrimination was observed, only a slight inversion of selectivity being obtained with the (*S*)-enantiomer of **1** (on the contrary, a more marked inversion had been observed[5] with the (*R*)-enantiomer of the analog **4**). To verify if the loss of selectivity was due to the presence of the naphthyl moiety, we compared the transformation of the 2-naphthaldehyde **2** with that of the natural substrate benzaldehyde **5**. As shown in the first two lines of Table 2, the results were almost equivalent. Finally, we compared the behaviors of the aldehydes **3** and **6**. The result obtained with 2-naphthylacetaldehyde **3** was quite unexpected, the enantiomeric ratio

Table 2
PaHNL-catalyzed transformation of aldehydes **2**, **3** and **5**, **6**

Substrate	Product	% Enantiomeric composition	
		2*R* (Xa)	2*S* (Xb)
2	**2a-b**	**97.6**	**2.4**
5	**5a-b**	**96.5**	**3.5**
3	**3a-b**	**33.2**	**66.8**
6	**6a-b**	**96.3**	**3.7**

Table 3
PaHNL-catalyzed transformation of aldehydes **7**–**10** and **14**, **15**

Substrate	Product	% Diastereomeric composition			
		2*S*, 3*S* (Xa)	2*R*, 3*S* (Xb)	2*S*, 3*R* (Xc)	2*R*, 3*R* (Xd)
7	–	**No transformation**			
8	**8a-d**	**43.1**	**8.4**	**40.1**	**8.4**
9	**9a-d**	**28.6** [a)]	**21.5** [b)]	**28.7** [a)]	**21.1** [b)]
10	**10a-d**	**6.5 (2*S*, 4*S*)**	**45.2 (2*R*, 4*S*)**	**14.0 (2*S*, 4*R*)**	**34.3 (2*R*, 4*R*)**
14	**14a-d**	**45.4**	**8.1**	**33.3**	**13.3**
15	**15a-d**	**26.9** [a)]	**23.4** [b)]	**29.2** [a)]	**20.6** [b)]

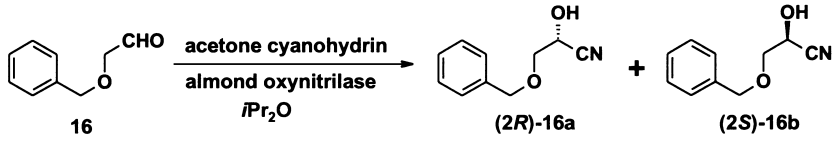
[a)] Values might be exchanged. [b)] Values might be exchanged.

being in favor of the 'wrong' (*S*)-enantiomer with the highest discrimination observed so far in favor of the unnatural stereoisomer (2:1 the ratio of **3b** over **3a**).

We then moved our attention to α- or β-oxygenated aldehydes. Table 3 show the results obtained with the racemic aldehydes **7**–**10**, **14**, **15** (isolated yields of the corresponding cyanohydrins were between 40 and 80%). It has to be noted again that, at variance with the usual PaHNL-catalyzed formation of (2*R*)-cyanohydrins, formally the (2*S*)-stereoisomers are produced from α-oxygenated aldehydes due to the CIP priority rules.

The data of Table 3 clearly show that the presence of an oxygen atom in the vicinity of the aldehydic moiety influences the selectivity of PaHNL. Specifically, the diastereomeric ratio of the different cyanohydrins was always worse than that obtained with the corresponding alkylated aldehydes **4**, **11**–**13** (Scheme 2).[1] For instance, while the aldehyde **9** gave the four cyanohydrins **9a**–**d** in an almost equimolecular ratio (28.6, 21.5, 28.7, 21.1) the data obtained with the deoxygenated analog **12** were 4.6% ((2*S*,3*S*)-diastereoisomer **12a**), 44.5 (2*R*,3*S*), 4.2 (2*S*,3*R*), and 46.7 (2*R*,3*R*), respectively.[1] α- and β-substituents seem to have similar effects (lines 2 and 4 of Table 3) and lower selectivity was observed with the (*R*)-enantiomer of the β-oxygenated aldehyde **10**.

It has been reported[13] that benzyloxyacetaldehyde **16**, a demethylated analog of **14**, was transformed without selectivity by the (*S*)-oxynitrilase from *Hevea brasiliensis*, the two enantiomeric cyanohydrins **16a** and **16b** being formed in an almost equimolecular ratio. We submitted **16** (Scheme 7) and its analog aldehydes **17**, **18** to the catalytic action of PaHNL, and the enantiomeric composition of the corresponding cyanohydrins is reported in Table 4.



Scheme 7.

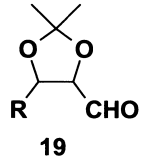
Table 4
PaHNL-catalyzed transformation of aldehydes **16**–**18**

Substrate	Product	% Enantiomeric composition	
		2*R* (Xa)	2*S* (Xb)
16	16a-b	43.9	53.1
17	17a-b	74.3	25.7
18	18a-b	88.9	11.1

The result obtained with benzyloxyacetaldehyde **16** was also quite intriguing, as the enzymatic selectivity was completely lost (Table 4, line 1). By moving the oxygen atom one carbon away (aldehyde **17**), the selectivity was partially restored (3:1 enantiomeric ratio in favor of the (*R*)-enantiomer) and, again, higher selectivity obtained with the deoxygenated analog **18** (9:1 enantiomeric ratio).

The above data are not easily rationalizable. The tridimensional structure of PaHNL has not been elucidated yet and the mechanism of action of this class of biocatalysts has not been completely clarified even for the proteins whose molecular structures are known.[3,14] It is clear that the presence of an oxygen atom in the vicinity of the carbonyl moiety as well as an increasing of the substituents dimensions is disturbing the correct positioning of the substrate into the active site, but no further information can be obtained at the molecular level.

Our future work will focus on other series of oxygenated (i.e. **19**) and 'bulkily' substituted aldehydes (i.e. **20**), as well as on the influence of other heteroatoms (nitrogen, sulfur) on PaHNL selectivity. Additionally, the substrates investigated so far will also be submitted to the action of the (*S*)-oxynitrilase obtained from *Hevea brasiliensis*.



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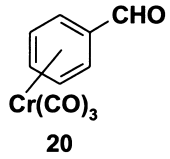

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3. Experimental

3.1. Materials and methods

Oxynitrilase was isolated from ground almonds.[15] Lipase PS from *Pseudomonas cepacia* was purchased from Amano. Acetone cyanohydrin and other reagents were from Aldrich. 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). Compounds **1a–d**, eluent: hexane:*i*PrOH, 95:5; flow rate: 0.5 ml/min; ret. time (min): **1a**, 60.36; **1b**, 73.23; **1c**, 78.09; **1d**, 56.36. Compounds **2a**,**b**, eluent: hexane:*i*PrOH, 95:5; flow rate: 0.5 ml/min; ret. time (min): (*R*)-**2**, 44.60; (*S*)-**2**, 53.92. Compounds **3a**,**b**, eluent: hexane:*i*PrOH, 95:5; flow rate: 0.5 ml/min; ret. time (min): (*R*)-**3**, 45.74; (*S*)-**3**, 49.68. Compounds **7a–d**, eluent: hexane:*i*PrOH, 97:3; flow rate: 0.7 ml/min; ret. time (min): **7a–d**, 33.93, 37.33, 44.32, 48.78. Compounds **8a–d**, eluent: hexane:*i*PrOH, 96:4; flow rate: 0.5 ml/min; ret. time (min): **8a**, 33.19; **8b**, 42.41; **8c**, 53.84; **8d**, 46.06. Compounds **9a–d** (as acetates), eluent: hexane:*i*PrOH, 95:5; flow rate: 1 ml/min; ret. time (min): **9a** and **9c**, 36.97, 52.52; **9b** and **9d**, 48.24, 59.29. Compounds **14a–d**, using two Chiralcel OD columns in series; eluent: hexane:*i*PrOH, 96:4; flow rate: 0.5 ml/min; ret. time (min): **14a**, 45.08; **14b**, 52.58; **14c**, 33.08; **14d**, 97.58. Compounds **15a–d** (as butanoates), using two Chiralcel OD columns in series; eluent: hexane:*i*PrOH, 98:2; flow rate: 0.5 ml/min; ret. time (min): **15a** and **15c**, 55.58; 57.83; **15b** and **15d**, 52.58, 60.08. Compounds **16a**,**b**, eluent: hexane:*i*PrOH, 98:2; flow rate: 1 ml/min; ret. time (min): (*R*)-**16**, 19.78; (*S*)-**16**, 23.38. Compounds **18a**,**b** (as acetates), eluent: hexane:*i*PrOH, 95:5; flow rate: 0.5 ml/min; ret. time (min): (*R*)-**18**, 25.77; (*S*)-**18**, 24.34.

GC analyses were performed using a Chrompack capillary column w cot fused silica gel coated with CP-cyclodex B236 M and a Hewlett Packard 5890 series II instrument. Compounds **10a–d** (as acetates), T_i=110°C; t_i=3 min; rate *A*=0.5°C/min; T_f*A*=135°C; rate *B*=0.2°C/min; T_f*B*=160°C; ret. times (min): **10a**, 107.739; **10b**, 109.277; **10c**, 110.550; **10d**, 106.227. Compounds **17a**,**b** (as acetates), T_i=140°C; t_i=1 min; rate=0.3°C/min; T_f=190°C; ret. times (min): (*R*)-**17**, 68.538; (*S*)-**17**, 67.361.

^{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

2-Naphthaldehyde **2** and benzyloxyacetaldehyde **16** are commercially available (Aldrich). Racemic 2-(2-naphthyl)propanal **1**[16] was prepared from 2′-acetonaphthone in analogy to a published procedure.[17] (a) Under inert atmosphere at room temperature, methoxymethyltriphenylphosphonium chloride (3.021 g, 8.81 mmol) was added to a solution of 2′-acetonaphthone (1 g, 5.875 mmol) in anhydrous toluene (20 ml). *Tert*-BuOK was added, divided into four parts, every 15 min. By adding *tert*-BuOK, the phosphonium salt dissolved and the reaction mixture turned to an orange color. The reaction was stirred until the mixture became deeply red and clear (3 h). When the reaction was completed (TLC: hexane:AcOEt, 8:2), the mixture was dropped in H_2O (50 ml), stirred for 10 min and then extracted with AcOEt. The extracts were dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (hexane:AcOEt, 8:2) gave the vinyl ether intermediate in 90% yield. (b) At 0°C a 1N solution of HCl (30 ml) was added in five portions every 10 min to a solution of the vinyl ether (1.1 g, 4.288 mmol) in THF (30 ml). EtOH (20 ml) was added to make the mixture homogeneous and the reaction was heated at 50°C (TLC: hexane:AcOEt, 8:2). After 6 h the reaction was cooled at room temperature and stirred overnight. Solid $NaHCO_3$ was added to neutralize the HCl and then the mixture was extracted with AcOEt. The extracts were dried (Na_2SO_4) and the solvent evaporated. Flash chromatography (hexane:AcOEt, 95:5) gave aldehyde **1** in 55% yield. The residue was purified with bulb-to-bulb distillation (150°C, 4 mmHg).

^{1}H NMR: δ=9.80 (s, 1H, CHO), 7.83 (m, 3H, ArH), 7.68 (s, 1H, ArH-1), 7.48 (m, 2H, ArH), 7.32 (m, 1H, ArH), 3.80 (q, 1H, *J*=8.3 Hz, H-2), 1.55 (d, 3H, *J*=8.3 Hz, CH_3).

(2-Naphthyl)ethanal **3**[16] was prepared from the corresponding alcohol using the NaOCl/TEMPO procedure.[7] At 0°C and under vigorous stirring a solution of NaOCl (15 ml of a 1:1 mixture of H_2O and of a 5% w/v NaOCl solution) was added dropwise to a solution of 2-(2-naphthyl)ethanol (1 g, 5.8 mmol), NaBr (600 mg, 5.8 mmol), TEMPO (30 mg, catalytic amount) in toluene (20 ml), AcOEt (20 ml), H_2O (5 ml). Following each addition the mixture became orange and then returned to a colorless state. When the addition of the solution of NaOCl was complete, the mixture was stirred at room temperature (TLC: hexane:AcOEt, 8:2). The two phases were separated: the aqueous phase was extracted with AcOEt and the combined organic phases were washed with a 10% solution of KI in $KHSO_4$ (0.5 ml, the organic phase became orange), then with a 10% solution of $Na_2S_2O_3$ (0.5 ml, the organic phase returned to a colorless state), with phosphate buffer (pH 7) and finally with brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated (yield 70%). The residue was purified with bulb-to-bulb distillation (150°C, 4 mmHg). ^{1}H NMR: δ=9.85 (t, 1H, *J*=2.0 Hz, CHO), 8.00–7.00 (m, 7H, ArH), 3.85 (d, 2H, *J*=2.0 Hz, H-2).

Methoxyphenylacetaldehyde **7**[18] was prepared from methyl mandelate, which was alkylated with CH_3I in the presence of NaH and reduced with 1 M solution of DIBAL in THF to the corresponding aldehyde in 30% yield. The residue was purified with bulb-to-bulb distillation (100°C, 4 mmHg). ^{1}H NMR: δ=9.60 (s, 1H, CHO), 7.30–7.50 (m, 5H, ArH), 4.75 (s, 1H, H-2), 3.40 (s, 3H, OMe).

2-Methoxy-3-phenylpropanal **8**[18] and 2-benzyloxy-3-phenylpropanal **15**.[19] Racemic **8** was prepared from 2-hydroxy-3-phenylpropanoic acid, which was esterified with MeOH saturated with gaseous HCl, alkylated with CH_3I in the presence of NaH and reduced with 1 M solution of $LiAlH_4$ in THF. The alcohol was then oxidized to the corresponding aldehyde with the Swern procedure.[6] At −60°C a solution of DMSO (1.2 ml, 15.9 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a 2 M solution of oxalyl chloride (4 ml, 8 mmol) in CH_2Cl_2. The mixture was stirred for 20 min and then a solution of 2-methoxy-3-phenylpropanol (1.1 g, 6.62 mmol) in CH_2Cl_2 was dropped. The mixture was stirred for 10 min and then Et_3N (4.6 ml, 33.13 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 organic phase was controlled by TLC (eluent: hexane:AcOEt, 8:2), washed with H_2O, 5% $NaHCO_3$, H_2O and dried (Na_2SO_4). The solvent was evaporated and the product was purified by flash chromatography (eluent: hexane:AcOEt, 9:1, yield 90%). The residue was purified with bulb-to-bulb distillation (100°C, 4 mmHg). ^{1}H NMR: δ=9.72 (d, 1H, *J*=2.3 Hz, CHO), 7.30 (m, 5H, ArH), 3.80 (ddd, 1H, J_1=6.9 Hz, J_2=5.2 Hz, J_3=2.3 Hz, H-2), 3.45 (s, 3H, OMe), 3.00 (m, 2H, CH_2-3).

Racemic **15** was prepared similarly using benzyl bromide instead of CH_3I as an alkylating agent. Swern oxidation of the corresponding alcohol gave **15** in 84% yield. The residue was purified with bulb-to-bulb distillation (120°C, 4 mmHg).

Racemic **9** was prepared following the procedure described in Scheme 3a. (a) At −78°C $LiAlH_4$ (3.4 ml of a 1.0 M solution in THF) was carefully dropped into a solution of 3-isochromanone (2 g, 13.5 mmol) in THF (20 ml). The reaction was stirred at −78°C for half an hour and then 0.5 equiv. (1.7 ml) of $LiAlH_4$ were additionally added. After half an hour TLC analysis (hexane:AcOEt, 6:4) showed the formation of a product with R_f 0.5 accompanied by a more polar by-product (about 10%). The reaction was stopped by adding AcOEt, and the mixture was dropped in water, acidified with HCl and extracted with AcOEt. The extracts were washed with $NaHCO_3$ and dried with Na_2SO_4, the solvent evaporated to give the aldehyde intermediate as a solid residue. ^{1}H NMR: δ=7.40–6.90 (m, 4H, ArH), 5.38 (t, 1H, *J*=4.5 Hz, H-3), 4.98 (d, 1H, *J*=14 Hz, H-1a), 4.78 (d, 1H, *J*=14 Hz, H-1b), 3.08 (dd, 1H, J_1=16.2 Hz, J_2=4.5 Hz, H-4a), 2.82 (dd, 1H, J_1=16.2 Hz, J_2=4.5 Hz, H-4b). (b) The solid residue was dissolved in 10 ml of CH_3NO_2,

$BuNH_2$ (500 μl) was added and the reaction was heated at 50°C for 6 h and stirred overnight (TLC: hexane:AcOEt, 8:2). The solvent was concentrated and the product was purified by flash chromatography (hexane:AcOEt, 9:1) to give 530 mg (40% yield) of the nitromethyl intermediate. ^{1}H NMR: δ=7.30–7.00 (m, 4H, ArH), 4.88 (s, 2H, CH_2-1), 4.55 (m, 3H, H-3 and CH_2-NO_2), 2.80 (d, 2H, J=5 Hz, CH_2-4). (c) Following catalytic hydrogenation (Pd/C–MeOH, overnight), 400 mg of the aminomethyl intermediate (2.45 mmol) were dissolved in CH_2Cl_2 (50 ml) and DMF (20 ml) under inert atmosphere. 4-Formyl-1-methylpyridiniumbenzenesulfonate[20] (Rapoport's reagent, 822 mg, 2.94 mmol) was added and the mixture was refluxed until the starting material disappeared (TLC: MeOH:AcOEt, 1:1). The reaction was cooled at 0°C and DBU (500 μl, 3.35 mmol) was added. The mixture, which turned from black to dark red, was stirred for 15 min and then, after adding a saturated solution of $H_2C_2O_4$ (50 ml), was stirred overnight. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried and the solvent evaporated. Flash chromatography (hexane:AcOEt, 7:3) gave 120 mg of **7** (yield 40%). The residue was purified with bulb-to-bulb distillation (130°C, 4 mmHg). ^{1}H NMR: δ=9.80 (s, 1H, CHO), 7.30–6.90 (m, 4H, ArH), 4.90 (2d, each 1H, J=15 Hz, H-1), 4.20 (t, 1H, J=7.5 Hz, H-3), 2.95 (d, 2H, J=7.5 Hz, CH_2-4).

Racemic 3-methoxy-3-phenylpropanal **10** was prepared from ethyl benzoylacetate, which was reduced to 3-phenyl-1,3-propandiol by action of $NaBH_4$ in EtOH at 0°C. The benzylic OH was selectively methylated by boiling in MeOH containing 1% concentrated H_2SO_4, while the primary OH was oxidized to aldehyde using the Swern procedure[6] to give **10** in 40% yield. The residue was purified with bulb-to-bulb distillation (100°C, 4 mmHg). ^{1}H NMR δ=9.80 (t, 1H, J=1.4 Hz, CHO), 7.35 (m, 5H, ArH), 4.68 (dd, 1H, J_1=8.5 Hz, J_2=4.2 Hz, H-3), 3.20 (s, 3H, OCH_3), 2.90 (ddd, 1H, J_1=15.7 Hz, J_2=11.4 Hz, J_3=2.9 Hz, H-2a), 2.63 (ddd, 1H, J_1=15.7 Hz, J_2=14.3 Hz, J_3=1.4 Hz, H-2b).

Racemic 2-benzyloxypropanal **14**[11] was prepared from methyl lactate, which was benzylated (benzyl bromide, NaH, THF), reduced to alcohol (1 M $LiAlH_4$ in THF) and oxidized to **14** using the Swern protocol (33% overall yield). The residue was purified with bulb-to-bulb distillation (150°C, 4 mmHg). ^{1}H NMR δ=9.70 (d, 1H, J=2 Hz, CHO), 7.40–7.20 (m, 5H, ArH), 4.70–4.55 (2d, each 1H, J=12 Hz, benzylic CH_2), 3.90 (dq, 1H, J_1=6.4 Hz, J_2=2 Hz, H-2), 1.35 (d, 3H, J=6.4 Hz, CH_3).

3-Phenoxypropanal **17**[21] and 4-phenylbutanal **18**[22] were prepared from the corresponding acid following the usual three-step protocol: esterification with MeOH saturated with gaseous HCl, reduction with a 1 M solution of $LiAlH_4$–THF and oxidation with the NaOCl/TEMPO procedure,[7] followed by purification by flash chromatography (eluent: hexane:AcOEt, 8:2, 9:1) and bulb-to-bulb distillation (**17**: bp=110°C, 4 mmHg; **18**: bp=100°C, 4 mmHg).

Compound **17**: ^{1}H NMR δ=9.90 (t, 1H, J=2.1 Hz, CHO), 7.40–6.80 (m, 5H, ArH), 4.32 (t, 2H, J=8.6 Hz, CH_2-3), 2.90 (m, 2H, J_1=8.6 Hz, J_2=2.1 Hz, CH_2-2). Compound **18**: ^{1}H NMR δ=9.78 (t, 1H, J=2 Hz, CHO), 7.25 (m, 5H, ArH), 2.67 (m, 2H, CH_2-4), 2.40–1.90 (m, 2H, CH_2-2), 1.75 (m, 2H, CH_2-3).

3.3. General procedure for the chemical synthesis of cyanohydrins

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 cyanohydrin mixtures (two enantiomers or four diastereoisomers) were purified by flash chromatography.

^{1}H NMR. Compounds **1a**–**d**: δ=8.00–7.30 (m, 7H, ArH), 4.60 (dd, 1H, J_1=8.6 Hz, J_2=6.8 Hz, H-2), 3.35 (quint, 1H, J=6.8 Hz, H-3), 1.60 (d, 3H, J=6.8 Hz, CH_3). Compounds **2a**–**d**: δ=8.02 (s, 1H, ArH), 7.95–7.82 (m, 3H, ArH), 7.62–7.52 (m, 3H, ArH), 5.72 (d, 1H, J=6.8 Hz, H-2), 2.25 (d, 1H, J=6.8 Hz,

OH). Compounds **3a**,**b**: ($CDCl_3$+D_2O) δ=8.00–7.20 (m, 7H, ArH), 4.75 (t, 1H, *J*=8 Hz, H-2), 3.30 (d, 2H, *J*=8 Hz, CH_2-3). Compounds **8a**–**d**: δ=7.25 (m, 5H, ArH), 4.25 (d, 1H, *J*=3.8 Hz, H-2), 3.63 (m, 1H, H-3), 3.51 and 3.48 (s, each 1.5H, OCH_3), 3.15 and 2.98 (dd, each 0.5H, J_1=14.2 Hz, J_2=6 Hz, H-4a), 2.88 and 2.82 (dd, each 0.5H, J_1=14.2 Hz, J_2=7.5 Hz, H-4b). Compounds **9a**–**d**: δ=7.30–7.00 (m, 4H, ArH), 4.97, 4.95, 4.89, 4.86 (4d, 2H, *J*=14 Hz, CH_2-1), 4.58 (d, 0.5H, *J*=4 Hz, C<u>H</u>-CN), 4.51 (d, 0.5H, *J*=5 Hz, C<u>H</u>-CN), 3.98 (m, 1H, H-3), 3.05 (m, 1H, H-4a), 2.82 (m, 1H, H-4b). Compounds **10a**–**d**: δ=7.35 (m, 5H, ArH), 4.75 (m, 1H, H-4), 4.42 (dd, 1H, J_1=6 Hz, J_2=3 Hz, H-2), 3.30 and 3.22 (s, each 1.5H, OCH_3), 2.35 and 2.08 (m, each 1H, H-3a, H-3b). Compounds **14a**–**d**: δ=7.45–7.25 (m, 5H, ArH), 4.73, 4.70, 4.66, 4.62 (4d, *J*=14 Hz, benzylic CH_2), 4.35 (2m, each 0.5H, H-2), 3.80 (2m, each 0.5H, H-3), 2.95–2.85 (2d, each 0.5H, *J*=7.5 Hz, OH), 1.35 (2d, each 1.5H, *J*=7.5 Hz, CH_3). Compounds **15a**–**d**: δ=7.30 (m, 5H, ArH), 4.68, 4.65, 4.62, 4.52 (4d, benzylic CH_2), 4.28 and 4.26 (2d, each 0.5H, *J*=4.3 Hz, H-2), 4.87 (m, 1H, H-3), 3.15 (dd, 0.5H, J_1=14 Hz, J_2=6.4 Hz, H-4a) and 3.00 (dd, 0.5H, J_1=12.9 Hz, J_2=6.4 Hz, H-4a), 2.92 (dd, 0.5H, J_1=14 Hz, J_2=7.5 Hz, H-4b), 2.78 (dd, 0.5H, J_1=12.9 Hz, J_2=9.7 Hz, H-4b). Compounds **16a**,**b**: δ=7.40 (m, 5H, ArH), 4.65 (s, 2H, benzylic CH_2), 4.55 (t, 1H, *J*=7 Hz, H-2), 3.75 (d, 2H, *J*=7 Hz, CH_2-3). Compounds **17a**,**b**: δ=7.40–6.80 (m, 5H, ArH), 4.83 (m, 1H, H-2), 4.28 (m, 2H, CH_2-4), 2.90 (d, 1H, *J*=6 Hz, OH), 2.33 (m, 2H, CH_2-3). Compounds **18a**,**b**: δ=7.25 (m, 5H, ArH), 4.45 (m, 1H, H-2), 2.70 (m, 2H, CH_2-5), 1.85 (m, 4H, CH_2-3 and CH_2-4).

3.4. General procedure for the enzymatic synthesis of the cyanohydrins

To a solution of 500 mg of aldehyde in 23 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 3–6 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.

3.5. General procedure for the acylation with lipase PS

About 30 mg/ml of substrate (alcohol or cyanohydrin) were dissolved in methyl-*tert*-butyl ether containing 10% v/v vinyl acetate and lipase PS immobilized on Celite[23] (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 evaporated and the products purified by flash chromatography.

3.6. Stereochemical correlations

(*S*)-2-(2-Naphthyl)propanal (*S*)-**1** was prepared from (*S*)-2-(2-naphthyl)propanol obtained by the corresponding racemate as described by Matsumoto et al.[9] (Scheme 5).

(*S*)-2-Methoxy-3-phenylpropanal (*S*)-**8** was prepared from the commercially available (*S*)-hydroxy acid (Scheme 5).

(*S*)-3-Methoxy-3-phenylpropanal (*S*)-**10** was prepared from ethyl (*S*)-3-hydroxy-3-phenylpropanoate, obtained as described by Santaniello et al.[10] (Scheme 5). Methylation was accomplished using the CH_3I/Ag_2O alkylating procedure,[24] and further elaboration of the molecule was achieved by $LiAlH_4$ reduction and NaOCl/TEMPO oxidation.

(*R*)-2-Benzyloxypropanal **14** was obtained by oxidation of the corresponding alcohol. In turn, the alcohol was obtained from a kinetic resolution of the racemate catalyzed by lipase PS (Scheme 5). The enzyme immobilized on Celite (1 g) was added to a solution of the racemic alcohol (4 g, 20.94 mmol)

in vinyl acetate (10 ml). The mixture was shaken at room temperature for 90 min (TLC: hexane:AcOEt, 7:3), then the enzyme was filtered off, the solvent evaporated and the residual alcohol was separated from the acetate by flash chromatography (hexane:AcOEt, 85:15, yield: 42%). The $[\alpha]_D$ of the alcohol was −39.0 (*c* 3, $CHCl_3$). In the literature it has been reported[11] that the (*S*)-alcohol has an $[\alpha]_D$ value of +43.1. Therefore, we isolated the (*R*)-enantiomer with 90.6 ee. The preferential acylation of the (*S*)-enantiomer is in agreement with the empirical rule suggested by Carrea et al.[25] for the esterification of primary alcohols catalyzed by lipase PS.

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