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The biocatalyzed stereoselective preparation of polycyclic cyanohydrins

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Abstract—The enzyme-mediated preparation of enantiomerically or diastereomerically enriched polycyclic cyanohydrins has been investigated. Oxynitrilase-catalyzed cyanurations gave excellent results with the bicyclic aldehydes tested. On the other hand, enantio- or diastereoselective acylation, catalyzed by lipase PS or subtilisin, proved to be a more versatile methodology, giving good results even with sterically hindered polycyclic cyanohydrins. Specifically, the steroidal cyanohydrin derivative **4b** was isolated with a 89% de.

1. Introduction

Enantiomerically or diastereomerically pure (or enriched) cyanohydrins are important synthetic intermediates that can be obtained either by chemical or enzymatic protocols.[1,2] To date, two biocatalyzed approaches have been developed. In the first method, the well-known substrate versatility of lipases has been exploited for the kinetic resolution of racemic cyanohydrin esters (by enantioselective hydrolysis or alcoholysis)[1,3,4] or of racemic cyanohydrins (by enantioselective acylation).[1,5] These biotransformations include one of the very first reports of enzymatic dynamic kinetic resolution, based on the easy in situ racemization of unreacted cyanohydrins under alkaline conditions.[6]

The development of enzymatic processes (up to multiton scale) for the oxynitrilases (HNLs)-mediated stereoselective addition of HCN to aldehydes or ketones[7] has become one of the most recent successes of biocatalysis.[1,2,8] Thanks mainly to Effenberger's and Griengl's contributions, new HNLs have been purified, cloned and over-expressed, and the reaction conditions have been optimized. Additionally, the structures of the (*R*)-oxynitrilase from almonds (PaHNL)[9] and of the (*S*)-oxynitrilases from *Hevea brasiliensis* (HbHNL),[10] *Manihot esculentia*[11] and *Sorghum bicolor*[12] have been solved by X-rays analysis, thus shedding light on the catalytic mechanism and selectivity displayed by these enzymes.

Recently, we have studied the performances of two oxynitrilases, PaHNL and HbHNL, towards a large set of α- and β-substituted aldehydes, focusing our attention on the influence of a pre-existing stereocentre on the selectivity displayed by these enzymes.[13] Herein we extend our investigation to the enzymatic cyanuration of the α-substituted steroidic aldehyde **1**, while addressing the more general problem of the biocatalyzed synthesis of enantiomerically enriched cyanohydrins of polycyclic aldehydes.

2. Results and discussion

The stereoselective elongation of the side chain of androstane- and pregnane-type steroids has attracted considerable attention, as it opens up synthetic routes to brassinolides, ecdysones and cholesterol derivatives.[14–17] In this respect, the diastereoselective synthesis of the

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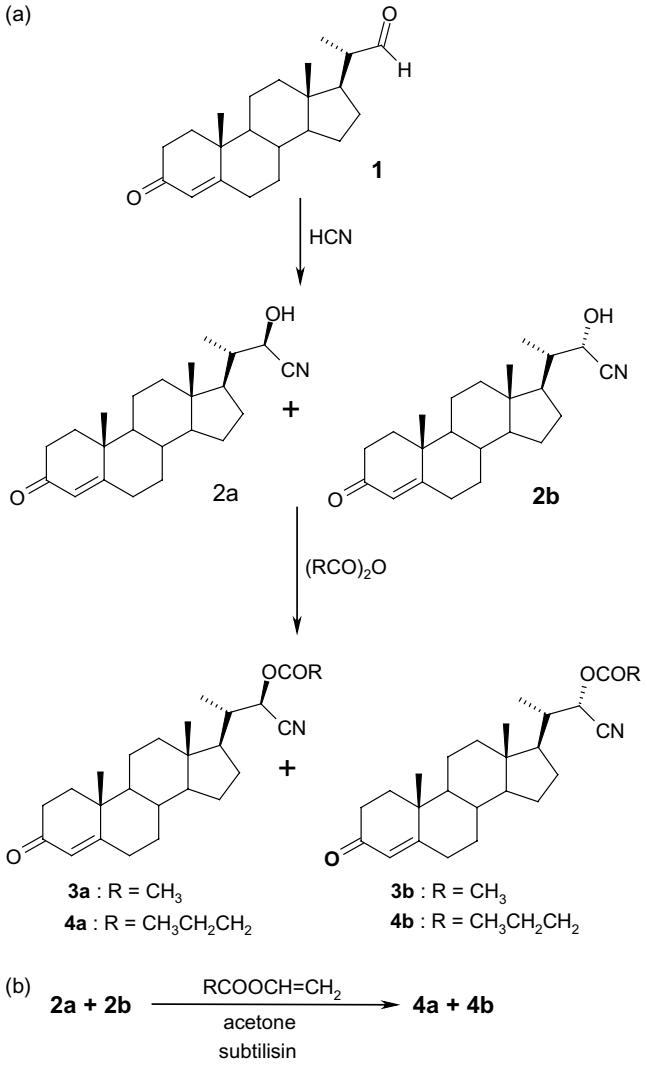
cyanohydrins **2a** or **2b** from the aldehyde **1**, which introduces a new stereocentre at C-22, could allow the asymmetric elaboration of the aliphatic side chain of this steroid.

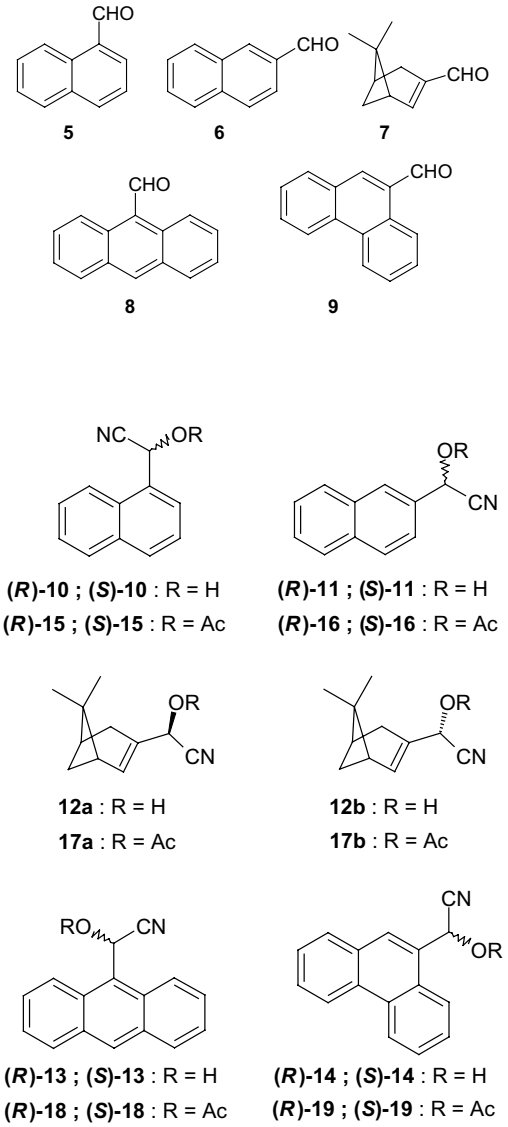
Chemical cyanuration of **1** gave the expected 1:1 mixture of the two C-22 epimers **2a** and **2b** (Scheme 1, part a). These diastereoisomers could not be separated by silica chromatography, however the ^{1}H NMR spectrum of their mixture showed base-line separated signals due to their H-22 (two doublets at 4.59 and 4.57 ppm) and CH_3-18 (two singlets at 0.76 and 0.75 ppm) protons. Additionally, **2a** and **2b** could be easily separated by a chiral HPLC column, thus assuring two independent analytical methods for the evaluation of the diastereomeric excess of the products obtained by HNLs catalysis.



Scheme 1.

However, enzymatic cyanuration of **1** using an optimized protocol[13] proved unsatisfactory: while both PaHNL and HbHNL accepted **1** as a substrate and catalyzed the synthesis of the respective cyanohydrins, but the reaction times were disappointingly long and no stereoselectivity was observed since the product was, with both oxynitrilases, an equimolar mixture of **2a** and **2b**.

Before tackling the diastereoselective preparation of the target cyanohydrin with an alternative approach, we analyzed the behaviour of PaHNL and HbHNL towards a series of polycyclic aldehydes, in order to get more detailed information on the substrate specificity of these enzymes towards these bulky substrates. The commercially available compounds **5**–**9** were submitted to the action of the oxynitrilases, with the corresponding products [from (***R***)- and (***S***)-**10** to (***R***)- and (***S***)-**14**] being isolated, characterized, and their ee (or de in the case of **12a** and **12b**) evaluated by chiral GC or HPLC.



As summarized in Table 1, the bicyclic aldehydes **5**–**7** were well accepted by both lipases, giving the corresponding cyanohydrins with high ee or de. Incidentally, an enantioselective synthesis of cyanohydrins **11** from

the aldehyde **6** has recently been explored[4] with the aim of preparing a chiral reagent for NMR shift. This was achieved by lipase-mediated resolution of the racemic cyanohydrin acetates **16**. Herein, confirming our previous reports,[13a,c] we have shown the effective oxynitrilases-mediated cyanuration of **6**, which renders, either of the enantiomers (***R***)-**11** and (***S***)-**11** in high yields and ee. Also worthy of note are the results obtained with (1*R*)-(−)-myrtenal **7**. This aldehyde proved to be a very good substrate for both HNLs, giving the corresponding epimeric cyanohydrin with extremely high de (no detection of the respective 'wrong' enantiomer was observed by GC, see Table 1).

Table 1. Enzymatic cyanuration of the polycyclic aldehydes **5**–**9**

Substrate	Enzyme	Conversion[a]	Product	Ee
5	PaHNL	89	**10a**–**b**	90.0 (*R*)
5	HbHNL	97	**10a**–**b**	73.4 (*S*)
6	PaHNL	>99	**11a**–**b**	95.2 (*R*)
6	HbHNL	85	**11a**–**b**	82.5 (*S*)
7	PaHNL	70.5	**12a**–**b**	>99.5 (*R*)[b]
7	HbHNL	63.2	**12a**–**b**	>99.5 (*S*)[b]
8	PaHNL	n.r.	**13a**–**b**	—
8	HbHNL	8	**13a**–**b**	0
9	PaHNL	n.r.	**14a**–**b**	—
9	HbHNL	6	**14a**–**b**	0

[a] Determined by GC.
[b] Diastereomeric excess of the new stereogenic centre.

In contrast, the two tricyclic aromatic aldehydes **8** and **9** were not substrates for these enzymes: apparently both HNLs seem to dislike polycyclic substrates. Whether this might be due to the bulkiness of the substrates, that prevents them reaching the enzyme active site (as in the case of **8** and **9**, in which the aldehyde moiety is directly linked to the polyaromatic skeleton) or to correctly position in it (the side chain aldehyde moiety of the tetracyclic steroid **1**), or to the hydrophobicity of these substrates, that prevents the partitioning of **8** and **9** in the water phase surrounding the enzymes, is a problem that deserves a more detailed investigation, possibly by molecular docking experiments based on the known 3-D structures of these proteins.[9,10]

As the enzymatic cyanuration of the tricyclic aldehydes was either unsuccessful (with **8** and **9**) or unsatisfactory (as with our target compound **1**), we turned our attention to the hydrolase-catalyzed kinetic resolutions of the racemates **13**, **14** and of the epimeric mixture **2a**–**b**.

The ability of lipase PS to catalyze the enantioselective acylation of (*S*)-cyanohydrins is well known[1] and, accordingly, the stereoselective esterification of racemic **13**, **14** in methyl *t*-butyl ether occurred with satisfactory enantioselectivity even under nonoptimized conditions, the *E*-values[18] being 49 and 17, respectively, [(***S***)-**18**, 91.7% ee, (***S***)-**19**, 76.2% ee]. Incidentally, while the synthesis of racemic **13** has been described before,[19] to the best of our knowledge, this is the first report on the preparation of enantiomerically enriched **13**, **14**, **18** and **19**.

The enzymatic discrimination of the epimers **2a**–**b** proved to be a more difficult task. Enzymatic acylation or deacylation of hydroxylated steroids have been investigated by us[20] and by others[21] in the past. As a general trend, it was found that lipases direct their action towards the OH's linked to the A-ring of steroids, while the protease subtilisin prefers the D-ring-linked hydroxyls. As far as the acylation of side chain hydroxyls concerned, there are reports related both on lipases (from *Candida cylindracea*,[21a] *Candida antarctica*[20c] and *Pseudomonas cepacia*[21d,e,f]) and on subtilisin.[20a] Attempted esterification of the cyanohydrins **2a**–**b** by catalysis of a set of lipases proved unsuccessful and the substrates were recovered unreacted. Literature reports are all related to the acylation of side chain primary hydroxyls,[20c,21a,d,e] with one exception, the diastereoselective esterification of 25-hydroxy-27-norcholesterol.[21f] All the steroidal substrates studied in those reports are sterically less hindered than **2a**–**b**, which may be the reason for the inability of the same lipases to modify our target compounds. However, subtilisin[20a] proved to be a suitable catalyst for the diastereoselective discrimination of the cyanohydrins **2a**–**b** (Scheme 1, part b). Different preparations of this enzyme were tested using THF as the solvent and vinyl acetate as the acylating agent, and better results were obtained using subtilisin CLECs®.[22] As shown in Table 2, further optimization of the reaction conditions indicated acetone and vinyl butanoate as a more suitable solvent and acyl donor, respectively. Under these conditions, the reaction was scaled up and the butanoate esters **4a**–**b** isolated in 89.5 de, as determined by chiral HPLC. Analysis of the ^{1}H NMR spectrum of the acylated product, using $CDCl_3$ as a solvent, showed the preferential formation of the compound possessing the H-22 signal as a doublet at 5.40 ppm, with a coupling constant of 2.14 Hz (the H-22 signal of the epimer was found as a minor doublet at 5.43, with a coupling

Table 2. Optimization of the reaction conditions for the kinetic resolution of the epimeric mixture **2a**–**b**

Subtilisin preparation	Solvent	Acylating agent	Reaction time (days)	Conversion (%)	$De_{(S)}$ (%)	$De_{(P)}$ (%)	E[a]
Chiro-CLEC	THF	Vinyl acetate	9	3.2	0.31	65.0	4.8
Pepti-CLEC	THF	Vinyl acetate	8	4.4	0.74	75.6	7.5
Chiro-CLEC	Acetone	Vinyl acetate	7	13.4	10.2	81.6	11.2
Pepti-CLEC	Acetone	Vinyl acetate	7	19	15.7	81.3	11.7
Chiro-CLEC	Acetone	Vinyl butanoate	10	30.7	35.1	89.0	25.3
Pepti-CLEC	Acetone	Vinyl butanoate	14	47.1	67.9	89.5	44

[a] *E* values were calculated from the de of the product[18].

constant of 3.78 Hz). In order to have an idea on the absolute configuration at the C-22 of the product, the structures of compounds **2a**–**4a** and **2b**–**4b** were submitted to molecular modelling analysis. Calculations were carried out in vacuum and in a $CHCl_3$ simulating environment, using the MacroModel 6.5 program[23] and the MMFF force field.[24] The minimized structures were almost equivalent in both the simulated environment and have been used to evaluate the respective coupling constants between H-21 and H-22,[25] obtaining the following results: **2a**, $J_{21-22} = 3.2$ Hz; **2b**, $J_{21-22} = 0.9$ Hz; **3a**, $J_{21-22} = 3.0$ Hz; **3b**, $J_{21-22} = 1.1$ Hz; **4a**, $J_{21-22} = 3.0$ Hz; **4b**, $J_{21-22} = 1.0$ Hz. This data is in qualitative agreement with the experimental J values, and therefore strongly suggest that subtilisin has a stereopreference for the acylation of the cyanohydrin with the smaller J_{21-22} value, that is for the C-22 (*S*)-epimer **2b**. The observed enzymatic stereoselectivity is of interest for synthetic applications, as several natural steroid derivatives, like α-ecdysone,[16] depresesterol,[15] or the recently isolated metabolites orthosterol B[26] and petuniasterone D,[27] possess a secondary OH with the same configuration at their C-22 (formally they are (*R*)-OH's due to the CAP priority rules). The elaboration of the nitrile moiety of the cyanohydrin **2b** (or of its ester derivative **4b**) can therefore open up the way to the stereoselective preparation of the side chain of these compounds.

3. Conclusion

The enzymatic preparation of enantiomerically or diastereomerically enriched cyanohydrins of polycyclic aldehydes by direct cyanuration or by selective acylation of the corresponding 'racemic' cyanohydrins has been investigated. HCN addition catalyzed by HNLs gave good results only with the bicyclic aldehydes **5**–**7**, while the biocatalyzed enantio- or diastereoselective acylation of 'racemic' cyanohydrins proved to be a more versatile methodology that can give good results even with sterically hindered substrates. Additionally, thanks to the larger number of available hydrolases, the search for a suitable biocatalyst can be more successful, as it has been exemplified with the cyanohydrins of our main target **1**. Despite the fact that the usual lipases were uneffective, it was possible to get a satisfactory enzymatic discrimination of **2a** and **2b** via the action of the protease subtilisin under optimized reaction conditions.

4. Experimental

4.1. General

Prunus amygdalus oxynitrilase (*PaHNL*) was isolated from grounded almonds.[28] The oxynitrilase from *H. brasiliensis* (*HbHNL*) was a gift from Prof. H. Griengl (Graz Technical University). Lipase PS from *P. cepacia* was purchased from Amano. CLEC®-subtilisin preparations was a gift from Altus Biologics Inc. Celite® R-630 was from Fluka. Acetone cyanohydrin and other reagents were from Aldrich. A ~0.6 M HCN solution in isopropyl ether was prepared according to Brussee.[29] Isopropyl ether used for enzymatic cyanurations was presaturated with 0.1 M citrate buffer pH 5. HPLC analyses were performed using either a Chiralcel OD (OD) or a Chiralcel OJ (OJ) 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. ^{1}H NMR spectra were recorded on a Bruker AC-300 at 300 MHz using $CDCl_3$ as a solvent.

4.2. Adsorption of lipase PS on Celite

Lipase PS (3 g) was mixed accurately with Celite® R-630 (10 g). Then, 10 mL of 0.1 M potassium phosphate buffer (pH 7) were added, the mixture vigorously shaken, spread on a Petri disk and left to dry at room temperature for 2 days.

4.3. Separation of enantiomeric (or epimeric) cyanohydrins and acylated cyanohydrins by chiral GC or chiral HPLC columns

HPLC. **2a**–**b**: OD column; eluent petroleum ether, isopropanol 85:15; flow rate 0.7 mL/min; $\lambda = 241$ nm; t_R (min): **2a**, 17.6; **2b**, 19.6. **3a**–**b**: OD column; eluent petroleum ether, isopropanol 85:15; flow rate 0.7 mL/min; $\lambda = 241$ nm; t_R (min): **3a**, 21.5; **3b**, 25.3. **4a**–**b**: OJ column; eluent petroleum ether–isopropanol 95:5; flow rate 1.0 mL/min; $\lambda = 241$ nm; t_R (min): **4a**, 23.7; **4b**, 29.1. (*R*)-**10** and (*S*)-**10**: OD column; eluent petroleum ether, isopropanol 93:7; flow rate 1.0 mL/min; $\lambda = 254$ nm; t_R (min): (*R*)-**10**, 27.1; (*S*)-**10**, 38.3. (*R*)-**11** and (*S*)-**11**: OD column; eluent petroleum ether, isopropanol 8:2; flow rate 0.85 mL/min; $\lambda = 254$ nm; t_R (min): (*R*)-**11**, 8.43; (*S*)-**11**, 9.43. (*R*)-**13** and (*S*)-**13** and (*R*)-**18** and (*S*)-**18**: OD column; eluent petroleum ether, isopropanol 7:3; flow rate 1.0 mL/min; $\lambda = 254$ nm; t_R (min): (*R*)-**13**, 9.2; (*S*)-**13**, 15.2; (*R*)-**18**, 6.5; (*S*)-**18**, 11.2. (*R*)-**14** and (*S*)-**14**: OD column; eluent petroleum ether, isopropanol 7:3; flow rate 1.0 mL/min; $\lambda = 254$ nm; t_R (min): (*S*)-**14**, 52.4; (*R*)-**14**, 65.8. (*R*)-**18** and (*S*)-**19**: OJ column; eluent petroleum ether–isopropanol 7:3; flow rate 0.6 mL/min; $\lambda = 254$ nm; t_R (min): (*S*)-**19**, 25.7; (*R*)-**19**, 30.4.

GC: (*R*)-**16** and (*S*)-**16**: $T_i = 110$ °C; $t_i = 1$ min; rate = 1.0 °C/min; $T_f = 200$ °C; t_R (min): (*R*)-**16**, 88.72; (*S*)-**16**, 89.86. **17a**–**b**: $T_i = 100$ °C; $t_i = 3$ min; rate = 1.0 °C/min; $T_f = 200$ °C; t_R (min): **17a**, 54.91; **17b**, 55.53.

4.4. Chemical synthesis of the epimeric cyanohydrins 22-hydroxy-3-oxo-pregn-4-ene-22-carbonitrile 2a and 2b

(a) To a solution of 3-oxo-pregn-4-ene-20β-carbaldehyde **1** (328 mg, 1 mmol) in ethanol (1 mL), acetone

cyanohydrin (642 μL, 7 equiv) and triethylamine (50 μL) were added. The vial was sealed with a rubber cap and the suspension stirred at room temperature. After 3 h, the TLC control indicated the disappearance of the starting material. The mixture was poured in water (20 mL), ice (20 g) and acetic acid (1 mL) under magnetic stirring. The precipitate was filtered, dried in a vacuum oven and the white powder obtained (320 mg, 90%) was characterized as the expected epimeric mixture of **2a** and **2b**.

(b) To a solution of **1** (200 mg, 0.61 mmol) in AcOEt–THF–80% AcOH (5 mL, 2:1:2 ratio), a solution of sodium cyanide (90 mg, 1.8 mmol) in 0.8 mL H_2O was added. The vial was sealed with a rubber cap and the suspension stirred at room temperature. After 5 h, the TLC control indicated the disappearance of the starting material. The solvent was evaporated and the residue purified by flash chromatography (eluent $CHCl_3$–MeOH 97:3) to give a white powder (193 mg, 94%) characterized as the expected epimeric mixture of **2a** and **2b**.

Melting point, 185.2–190.8 °C; TLC (eluent toluene–ethyl ether 7:3), $R_f = 0.31$; HPLC: the areas of the two peaks at 17.6 and 19.6 min presented a ratio of 48.5/51.5%; ^{1}H NMR ($CDCl_3$) δ: **2a**: 0.76 (3H, s, CH_3-18); 1.17–1.26 (6H, 1d and 1s, CH_3-21 and CH_3-19); 4.57 (1H, d, $J = 3.88$ Hz, H-22); 5.74 (1H, s, H-4). **2b**: 0.75 (3H, s, CH_3-18); 1.17–1.26 (6H, 1d and 1s, CH_3-21 and CH_3-19); 4.59 (1H, d, $J = 2.17$ Hz, H-22); 5.74 (1H, s, H-4). The integration of the two signals (singlet peaks) due to the C-18 methyl gave a 43.5/56.5% ratio for the two isomers. The integration of the two signals (doublet peaks) due to the C-22 proton gave a 47.9/52.1% ratio of the two isomers. ^{13}C NMR ($CDCl_3$) δ: (75.47 MHz) 64.79 and 65.09 (C-22); 118.52 and 120.04 (CN); 123.73 and 123.79 (C-4); 171.72 and 171.88 (C-5); 200.01 and 200.11 (C-3). IR (ATR): ν_{max} 3229.2 (OH, H bond); 2935 (C–H stretching); 2858.9; 1654.6 (C=O); 1609.3 (C=C stretching); 1432.8 (CH_2 bending); 1064.5 (C–O) cm^{-1}.

4.5. Attempted enzymatic cyanuration of 1

4.5.1. Using HCN. (a) PaHNL (~150 units), dissolved in 50 μL of 0.1 M citrate buffer pH 5.5, was dropped homogeneously on Celite (150 mg). The catalyst was added to 1.5 mL of isopropyl ether–DMF (4:1) containing 41 mg of **1** and 2 equiv of HCN, and the reaction shaken at room temperature at 150 rpm for 2 days, following the conversion by TLC. A blank reaction, performed in the presence of Celite without the enzyme, showed a significant percentage of spontaneous cyanuration. At the end of the reaction, the mixture was filtered, the Celite washed with isopropyl ether and the organic phases evaporated. The cyanohydrins were purified by flash chromatography (eluent $CHCl_3$–MeOH 97:3) to give 25 mg of **2a**–**b** (0.07 mmol, 58%). HPLC analysis (see Section 4.3) showed an equimolar ratio of the peaks due to **2a** and **2b**.

(b) HbHNL (~150 units), that had been dissolved in 50 μL of 0.1 M citrate buffer pH 5.5, was dropped homogeneously on Celite (150 mg). The catalyst was added to 1.5 mL of isopropyl ether–DMF (4:1) containing 41 mg of **1** and 2 equiv of HCN, and the reaction shaken at room temperature at 150 rpm for 2 days, following the conversion by TLC. A blank reaction, performed in the presence of Celite without the enzyme, showed a significant percentage of spontaneous cyanuration. At the end of the reaction the mixture was filtered, the Celite washed with isopropyl ether and the organic phases were evaporated. The cyanohydrins were purified by flash chromatography (eluent $CHCl_3$–MeOH 97:3) to give 30 mg of **2a**–**b** (0.084 mmol, 70%). HPLC analysis (see Section 4.3) showed an equimolar ratio of the peaks due to **2a** and **2b**.

4.5.2. Using acetone cyanohydrin. To a solution of 50 mg of aldehyde in 2.5 mL of isopropyl ether–DMF (4:1) containing 4 equiv of acetone cyanohydrin, PaHNL or HbHNL (150 units adsorbed on 150 mg Celite) was added and the biphasic system shaken at room temperature for 3 days, following the conversion by TLC. A blank reaction, performed in the presence of Celite without the enzyme, did not show any spontaneous and unspecific cyanuration. Usual work-up, followed by flash chromatography, gave **2a**–**2b** (40 mg, 84%, using PaHNL; 46 mg, 87%, using HbHNL). HPLC analysis (see Section 4.3) showed again an equimolar ratio of the peaks due to **2a** and **2b**.

4.6. Chemical cyanuration of aldehydes 5–9

Cyanohydrins (from racemic **10** to racemic **14**) were prepared following the procedure described in Section 4.4(b).

^{1}H NMR selected data ($CDCl_3 + D_2O$). (***R***)- and (***S***)-**10** δ: 6.13 (1H, s, CHCN); 7.47–7.63 (3H, m, ArH); 7.82, 7.92, 7.93 and 8.12 (1H each, d, $J = 8.7$ Hz, ArH). (***R***)- and (***S***)-**11** δ: 5.72 (s, 1H, CHCN); 7.52–7.62 (m, 3H, ArH); 7.82–7.95 (m, 3H, ArH); 8.02 (s, 1H, ArH). **12a**–**b** δ: 0.83 (3H, s, CH_3); 1.34 (3H, s, CH_3); 2.10–2.60 (m, aliphatic H); 4.82 (1H, s, CHCN); 5.88 (1H, br s, CH=C). (***R***)- and (***S***)-**13** δ: 6.88 (1H, s, CHCN); 7.43 and 7.54 (2H each, br t, $J = 8.5$ Hz, ArH); 7.96 and 8.51 (2H each, d, $J = 8.5$ Hz, ArH); 8.43 (1H, s, ArH). (***R***)- and (***S***)-**14** δ: 5.99 (1H, s, CHCN); 7.43–7.54 (4H, m, ArH); 7.79, 8.11, 8.52 and 8.60 (1H each, d, $J = 8.4$ Hz, ArH); 7.95 (1H, s, ArH).

4.7. Enzymatic cyanuration of aldehydes 5–9

~150 Units of the oxynitrilase (PaHNL or HbHNL), dissolved in 50 μL of 0.1 M citrate buffer pH 5.5, were dropped homogeneously on Celite (150 mg). The catalyst was added to 2.5 mL of isopropyl ether containing 0.3 mmol of substrate and 2 equiv of HCN. The reaction was shaken at room temperature at 150 rpm for 2 days. No appreciable conversions were observed by TLC in

the blank reactions performed without enzyme, either in the presence or absence of Celite. The substrates **8** and **9** remained unreacted. At the end of the reactions (degrees of conversion are reported in Table 1), the mixtures were filtered, the Celite washed with isopropyl ether and the organic phases evaporated. The cyanohydrins (***R***)- and (***S***)-**10**;[6,30] (***R***)- and (***S***)-**11** were purified by flash chromatography (recovered yields 50–60%) and analyzed by chiral HPLC (see Section 4.3). The cyanohydrins **12a** and **12b**, obtained from the respective oxynitrilase-catalyzed reaction, were chemically acetylated (Ac_2O + 4-dimethylaminopyridine) to give the corresponding acetates **17a** and **17b** (^{1}H NMR ($CDCl_3 + D_2O$): **17a**–**b** δ: 0.83 (3H, s, CH_3); 1.34 (3H, s, CH_3); 2.29 (3H, s, CH_3CO); 2.10–2.60 (m, aliphatic H); 5.75 (1H, s, CHCN); 5.93 (1H, br s, CH=C). ^{13}C NMR ($CDCl_3 + D_2O$): **17a**–**b** δ: 169.0; 138.8; 126.6; 115.3; 63.5; 42.8; 40.3; 38.1; 31.3; 25.8; 20.7; 20.4), that were analyzed by chiral GC (see Section 4.3).

4.8. Chemical acylation of compounds 2a,b

The cyanohydrins **2a**–**b** (177,8 mg, 0.5 mmol) were dissolved in pyridine (4 mL) after which acetic anhydride (118 μL, 2.5 equiv) or butyric anhydride (205 μL, 2.5 equiv) was added. The reaction was left overnight at room temperature, after which 2 M HCl was added and the mixture then stirred and extracted with ethyl acetate. The combined organic layers were washed with sodium bicarbonate solution and brine, dried over anhydrous sodium sulfate and finally evaporated. The products obtained were characterized as **3a**–**b** (white solid) and **4a**–**b** (oil), respectively.

3a–**b**: TLC (eluent: toluene–ethyl ether 7:3), $R_f = 0.42$. ^{1}H NMR ($CDCl_3$), selected data, δ: 0.74 (3H, s, CH_3-18); 1.18 (3H, s, CH_3-19); 1.19 and 1.25 (3H, d each, $J = 4,68$ and 6,83 Hz, respectively, CH_3-21); 5.38 and 5.41 (1H total, d each, $J = 2.19$ and 3.78 Hz, respectively, H-22); 5.73 (1H, s, H-4). The integration of the doublet peaks due to H-22 gave a 45.3/54.7% ratio of the two isomers. ^{13}C NMR ($CDCl_3$) δ: 64.79 and 65.09 (C-22); 115.4 and 116.9 (CN); 123.8 (C-4); 169.1 and 169.4 (C-5); 171.1 (CH_3C=O); 199.5 (C-3).

4a–**b**: TLC (eluent: toluene–ethyl ether 7:3), $R_f = 0.48$. ^{1}H NMR ($CDCl_3$), selected data, δ: **4a**: 0.75 (3H, s, CH_3-18); 0.97 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2CO$); 1.17–1.26 (6H, 1d and 1s, CH_3-21 and CH_3-19); 2.38 (2H, m, $CH_3CH_2CH_2CO$); 5.43 (1H, d, $J = 3.78$ Hz, H-22); 5.74 (1H, s, H-4). **4b**: 0.75 (3H, s, CH_3-18); 0.97 (3H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2CO$); 1.17–1.26 (6H, 1d and 1s, CH_3-21 and CH_3-19); 2.38 (2H, m, $CH_3CH_2CH_2CO$); 5.40 (1H, d, $J = 2.14$ Hz, H-22); 5.74 (1H, s, H-4). Integration of the two doublets peaks due to H-22 gave a 49.5/50.5 ratio of the two isomers. ^{13}C NMR ($CDCl_3$) δ: 64.53 and 65.06 (C-22); 115.54 and 116.97 (CN); 123.83 (C-4); 171.21, 171.24, 171.73 and 171.99 (C-5 and $CH_3CH_2CH_2CO$); 199.61 and 199.64 (C-3). IR (ATR): ν_{max} 2931.3 (C–H stretching); 2870; 1746 (C=O ester); 1661 (C=O ketone); 1610 (C=C stretching) cm^{-1}.

4.9. Lipase PS catalyzed transesterification of racemic 13

Racemic **13** (70 mg, 0.30 mmol) was dissolved in methyl-*t*-butyl ether (2 mL), after which vinyl acetate (2 mL) and Celite-immobilized lipase PS (300 mg) were added. The suspension was shaken at 45 °C for 48 h (43.8% conversion, as determined by chiral HPLC). The enzyme was removed by filtration, the resulting filtrate concentrated in vacuum and the products were isolated by flash chromatography on silica gel (eluent: petroleum ether–AcOEt 85:15) to give (***S***)-**18** (25 mg, 91.7 ee) and unreacted (***R***)-**13** (19 mg, 75.9 ee).

(***S***)-**18**. $[\alpha]_D^{25}$ +48.6 (*c* 1.23, $CHCl_3$). ^{1}H NMR ($CDCl_3 + D_2O$) δ: 2.20 (3H, s, CH_3CO); 7.57 and 7.70 (2H each, br t, $J = 8.5$ Hz, ArH); 8.05 (1H, s, CHCN); 8.09 and 8.55 (2H each, d, $J = 8.5$ Hz, ArH); 8.64 (1H, s, ArH). ^{13}C NMR ($CDCl_3 + D_2O$) δ: 169.0; 131.7; 131.4; 130.1; 129.5; 127.8; 125.5; 123.3; 121.2; 116.9; 56.8; 20.5.

4.10. Lipase PS catalyzed transesterification of racemic 14

Racemic **14** (100 mg, 0.43 mmol) was dissolved in methyl-*t*-butyl ether (4 mL), after which vinyl acetate (1 mL) and lipase PS (100 mg) were added. The suspension was shaken at 45 °C for 7 days (50.8% conversion, as determined by chiral HPLC). The enzyme was removed by filtration, the filtrate concentrated under vacuum and the products isolated by flash chromatography on silica gel (eluent: petroleum ether–AcOEt 85:15) to give (***S***)-**19** (48 mg, 76.2 ee) and unreacted (***R***)-**14** (61 mg, 77.3 ee).

(***R***)-**14**. $[\alpha]_D^{25}$ +39.7 (*c* 1.27, $CHCl_3$). ^{1}H NMR ($CDCl_3 + D_2O$) δ: 5.99 (1H, s, CHCN); 7.43–7.54 (4H, m, ArH); 7.79, 8.11, 8.52 and 8.60 (1H each, d, $J = 8.4$ Hz, ArH); 7.95 (1H, s, ArH). ^{13}C NMR ($CDCl_3$) δ: 130.7; 129.9; 129.2; 128.6; 127.7; 127.1; 126.6; 124.1; 123.3; 122.5; 119.7; 61.9.

4.11. PeptiCLEC-subtilisin catalyzed transesterification of 2a–b

In a scale up reaction, **2a**–**b** (125 mg, 0.35 mmol) was dissolved in acetone (9.5 mL) after which vinyl butanoate (2.5 mL, 19.2 mmol) and PeptiCLEC-subtilisin (10 mg) were added. The reaction was carried out in a thermostatic shaker at 45 °C and followed by TLC and by HPLC. After 7 days another 10 mg of PeptiCLEC-subtilisin were added and the reaction left for another 7 days (final conversion 43.1%—as determined by chiral HPLC). The enzyme was then filtered off, the filtrate concentrated under reduced pressure and the butanoate **4b** separated from the unreacted cyanohydrin by flash chromatography (eluent: toluene–diethyl ether 85:15) to give a white solid (36 mg, 24.1% yield) with a 89.5 de {$[\alpha]_D^{30}$ +87.7, (*c* 1.0, $CHCl_3$)} as determined by chiral HPLC. Unreacted **2a** (57 mg) presented a 67.9 de {$[\alpha]_D^{30}$ +83.2, (*c* 1.0, $CHCl_3$)} as determined by chiral HPLC.

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