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Asymmetric biocatalytic hydrocyanation of pyrrole carboxaldehydes

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Abstract—The asymmetric hydrocyanation of pyrrole-2- and -3-carboxaldehydes substituted with either methyl, benzyl or phenyl in the 1-position catalyzed by the hydroxynitrile lyases from *Hevea brasiliensis* (*Hb*HNL) and *Prunus amygdalus* (*Pa*HNL) is reported. The products could be isolated—after *O*-silylation—with moderate to good enantiomeric purity although the carbonyl activity of the substrates was found to be very low, which is supported by quantum-chemical calculations. Structural effects concerning substrate size and regiochemistry are discussed considering docking calculations based on the X-ray crystal structures of the two enzymes. From these calculations one particular amino acid residue (Trp-128) in the active site of *Hb*HNL could be identified, which plays a major role for the appropriate binding of structurally demanding carbonyl compounds.

1. Introduction

The enzyme mediated synthesis of enantiomerically enriched cyanohydrins from aldehydes and ketones has been extensively explored for many years.[1] These compounds are important synthetic intermediates for the production of valuable biologically active substances.[2]

Among hydroxynitrile lyases (HNLs), the biocatalysts that catalyze the addition of hydrogen cyanide to carbonyl compounds resulting in the formation of cyanohydrins, the HNLs from the tropical rubber tree (*Hevea brasiliensis*, *Hb*HNL, E.C. 4.1.2.39) and from almonds (*Prunus amygdalus*, *Pa*HNL, E.C. 4.1.2.10) belong to the most comprehensively studied enzymes of this class. Both are characterized by their enormously high substrate tolerance that ranges from saturated and unsaturated aliphatic to aromatic and heterocyclic aldehydes and ketones. In addition, the two aforementioned enzymes are enantio-complementary. *Hb*HNL preferentially catalyzes the synthesis of *S*-configured cyanohydrins whereas in the presence of *Pa*HNL the addition of cyanide to the carbonyl function occurs predominantly in an *R*-selective manner.[3]

Even though a wide range of different substrates have been converted successfully, within the group of heterocyclic aromatic aldehydes only furan-2- and -3-carboxaldehydes and thiophene-2- and -3-carboxaldehydes turned out to be suitable substrates for *Hb*HNL and *Pa*HNL.[1d,4] However, pyrrole-2-carboxaldehyde could not be converted to the corresponding cyanohydrin using HNLs.[4b,5]

Recently, this fact has been attributed to the N–H function of the pyrrole ring and several *N*-substituted derivatives of pyrrole-2-carboxaldehyde were subjected to the reaction with HCN in the presence of a *Pa*HNL preparation in organic solvents.[6]

As part of our efforts to extend the substrate spectrum of HNLs and to understand the influence of substrate structure on HNL-catalyzed hydrocyanations, we undertook a series of experiments investigating the addition of HCN to *N*-substituted pyrrole-2- and -3-carboxaldehydes under the catalytic action of both *Hb*HNL and *Pa*HNL.

Furthermore, to gain more detailed insight and to learn more about the binding mode of the examined cyanohydrins compared to mandelonitrile docking calculations were performed based on the recently elucidated X-ray crystal structures of *Hb*HNL and *Pa*HNL.[7]

These results allow for general conclusions concerning the

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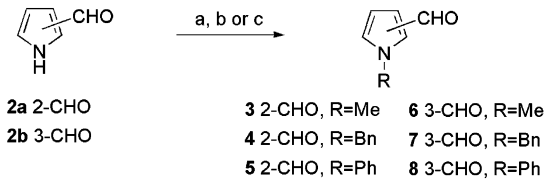
structural requirement of both enzymes regarding substrate size and regiochemistry.

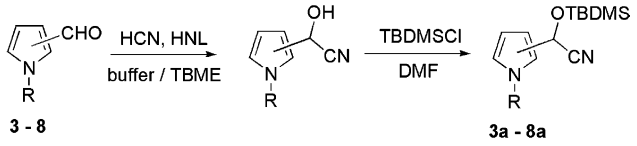
2. Results and discussion

Pyrrole-2- and -3-carboxaldehyde substituted at the 1-position with either methyl, benzyl, or phenyl were used as starting materials in this study.

Pyrrole-2-carboxaldehyde (**2a**) and *N*-methylpyrrole-2-carboxaldehyde (**3**) are commercially available. Pyrrole-3-carboxaldehyde (**2b**) was prepared by Vilsmeier-Haack formylation of *N*-triisopropylsilyl pyrrole (**1**), which was obtained by silylation of pyrrole with triisopropylsilyl chloride.[8] *N*-Methylpyrrole-3-carboxaldehyde (**6**) was obtained by methylation of **2b** using iodomethane in the presence of potassium *tert*-butoxide and 18-crown-6 in cyclohexane/DMSO.[9] Benzylation of both **2a** and **2b** was carried out by employing benzyl bromide and tetrabutylammonium bromide in dichloromethane and aqueous sodium hydroxide as the base.[10] Aldehydes **5** and **8** were synthesized from **2a** and **2b**, respectively, using phenylboronic acid in combination with copper (II) acetate and triethylamine (Scheme 1).[11]



Scheme 1. (a) CH_3I, KO*t*Bu, 18-crown-6, cyclohexane/DMSO; (b) benzyl bromide, TBABr, 50% aqueous NaOH, CH_2Cl_2; (c) phenylboronic acid, triethylamine, $Cu(OAc)_2$, CH_2Cl_2.

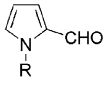
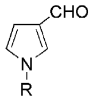


Scheme 2. Synthesis of protected cyanohydrins.

The enzyme catalyzed cyanohydrin reactions were carried out in a biphasic aqueous organic emulsion system (buffer/*tert*-butyl methyl ether).[4d] Since decomposition or racemization is likely to occur with cyanohydrins from aromatic aldehydes, all crude products were protected as TBDMS ethers without purification (Scheme 2). Analyses of the silylated cyanohydrins were performed either by chiral GC or by HPLC. The results obtained in the transformations catalyzed by either *Hb*HNL or *Pa*HNL5 (isoenzyme #5 of the HNL from almonds)[12] are summarized in Tables 1 and 2.

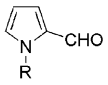
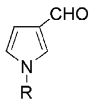
With both biocatalysts *N*-methylated derivatives **3** and **6** turned out to be more reactive than the *N*-benzylated aldehydes **4** and **7** even considering that more HCN was applied in the transformation of **3** compared to **4**. On the other hand *N*-benzylated pyrrole-carboxaldehydes were converted with better stereoselectivities in most cases. With *Hb*HNL both within the series of pyrrole-2- and -3-carboxaldehydes the protected cyanohydrins from the

Table 1. Synthesis of cyanohydrins from *N*-substituted pyrrole-2- and pyrrole-3-carboxaldehydes in the presence of the hydroxynitrile lyase from *H. brasiliensis*

									
	t^a (d)	Yield^b (%)	ee^b (%)	HCN (equiv)		t^a (d)	Yield^b (%)	ee^b (%)	HCN (equiv)
3 $_{R=Me}$	8	17	5	10	**6** $_{R=Me}$	11	64	40	10
4 $_{R=Bn}$	19	7	51	5	**7** $_{R=Bn}$	10	35	91	10
5 $_{R=Ph}$	15	67	0	5	**8** $_{R=Ph}$	21	0	—	10

[a] Corresponding to the enzymatic reaction.
[b] Determined after protection of the cyanohydrin with TBDMSCl.

Table 2. Synthesis of cyanohydrins from *N*-substituted pyrrole-2- and pyrrole-3-carboxaldehydes in the presence of the hydroxynitrile lyase from *P. amygdalus* (*Pa*HNL5, Isoenzyme #5)

									
	t^a (d)	Yield^b (%)	ee^b (%)	HCN (equiv)		t^a (d)	Yield^b (%)	ee^b (%)	HCN (equiv)
3 $_{R=Me}$	8	11	75	10	**6** $_{R=Me}$	11	49	82	10
4 $_{R=Bn}$	19	2	29	5	**7** $_{R=Bn}$	10	9	91	10
5 $_{R=Ph}$	15	11	0	5	**8** $_{R=Ph}$	21	0	—	10

[a] Corresponding to the enzymatic reaction.
[b] Determined after protection of the cyanohydrin with TBDMSCl.

N-benzylated derivatives could be isolated with higher enantiomeric excesses, whereas with *Pa*HNL5 in the series of pyrrole-2-carboxaldehydes **3a** was formed with higher enantiomeric purity than **4a**. Aldehydes carrying a phenyl substituent at the 1-position (**5** and **8**) turned out to be unsuitable substrates for both HNLs, since in the case of **5** only racemic cyanohydrin could be detected and **8** did not furnish a product at all.

Analyzing the substrates (**3**–**8**) with respect to the position of the carbonyl group, similar trends could be observed with both enzymes. *N*-substituted pyrrole-3-carboxaldehydes **6** and **7** furnished the corresponding cyanohydrins with higher yields and ee compared to the corresponding starting materials **3** and **4** (again considering different amounts of HCN with **4** and **7**).

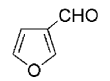
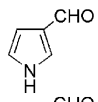
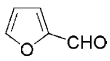
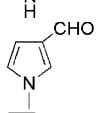
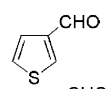
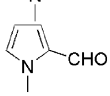
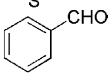
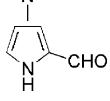
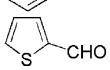
In general all substrates turned out to exhibit poor reactivities for cyanide addition leading to reaction times of several days. Quantum-chemical calculations of the reaction energy for the isodesmic reaction R-CHO + Ph-CH(O^-)–CN → R-CH(O^-)–CN + Ph-CHO indicate that pyrrole-carboxaldehydes are considerably less reactive towards addition of cyanide compared to benzaldehyde as well as furan- and thiophene-carboxaldehydes (Table 3). These findings are well in line with our experimental results. The lower reactivity of pyrrole- versus furan- and thiophene-carboxaldehydes can be made plausible by a consideration of the mesomeric forms with participation of the heteroatom lone pair taking into account the heteroatom electronegativity and is also evident from infrared data, which show significantly lower wave numbers of the carbonyl stretch mode.[13] Furthermore, the same trend can be seen from the pK_a values of the corresponding carboxylic acids (pyrrole > thiophene > furan).[14]

As mentioned before, pyrrole-3-carboxaldehydes as compared to the 2-isomers were found to be more suitable substrates for the hydrocyanation reactions catalyzed by the hydroxynitrile lyases from *H. brasiliensis* and *P. amygdalus*. This trend is already evident in the estimated relative reactivities of these aldehydes (Table 3) indicating a higher reactivity of **6** compared to **3**, and it is likely to assume that the position of the carbonyl function on the aromatic ring has a greater impact on the intrinsic reactivity of the aldehydes than the type of substituent attached to nitrogen. Secondly, the steric hindrance caused by the *N*-substituent is minimized in the case of the 3-isomers, which facilitates the appropriate binding of the aldehyde to the enzymes. In addition, the higher ee values of **4a** and **7a** (with the exception of **4a** in the *Pa*HNL5 series) can be ascribed to the decreased solubility of **4** and **7** compared to **3** and **6** in the aqueous phase resulting in a decreased rate of the unselective spontaneous HCN addition.

The corresponding cyanohydrins from aldehydes **3**–**8** were docked in silico to the active sites of *Hb*HNL and *Pa*HNL5. With the smaller cyanohydrins (**3a** and **6a**) these calculations revealed binding modes, which are virtually identical to the general binding mode of cyanohydrins observed experimentally.[7a] In the modeled complexes, the position of the pyrrole ring closely corresponds to the position of the phenyl ring in the respective complexes with mandelonitrile (Fig. 1).[15] In line with the known stereopreference of the two enzymes, *S*-configured cyanohydrins had more favourable calculated binding energies in the case of *Hb*HNL, whereas docking of the *R*-enantiomers resulted—in case of *Pa*HNL5—in lower energies. The respective energy differences between *R*- and *S*-enantiomers were comparable to those found in equivalent calculations with mandelonitrile.[15a] Thus, there appear to exist no steric reasons why pyrrole-carboxaldehydes (with no or small *N*-substituents) should not be accepted as substrates by *Hb*HNL and *Pa*HNL5. Therefore, the main reason for the unsatisfying results with substrates **3** and **6** seems to be—apart from the poor intrinsic carbonyl activity—the higher solubility in the aqueous phase and therefore increased unselective background reaction.

With larger cyanohydrins (*N*-benzyl and *N*-phenyl), no adequate binding modes to the active sites of both enzymes could be predicted in the calculations. For this reason amino acid residue Trp-128 in *Hb*HNL was replaced by alanine in silico (W128A). As a consequence of this artificial mutation, binding modes for the cyanohydrins from **4** and **7** were identified, which show all characteristic features observed for cyanohydrin binding, such as the interaction of the cyano group with amino acid residue Lys-236 and hydrogen bonds of the hydroxyl function with Ser-80 and

Table 3. Relative reactivities of several aromatic aldehydes estimated from the isodesmic reaction R-CHO + Ph-CH(O^-)–CN → R-CH(O^-)–CN + Ph-CHO

Entry	Structure	ΔE (kcal/mol)	Entry	Structure	ΔE (kcal/mol)
1		−3.9	6		2.4
2		−1.9	7		2.4
3		−1.5	8		3.2
4		0.0	9		4.1
5		0.2			

Negative values indicate a higher reactivity, positive values indicate a lower reactivity towards addition of cyanide compared to benzaldehyde.

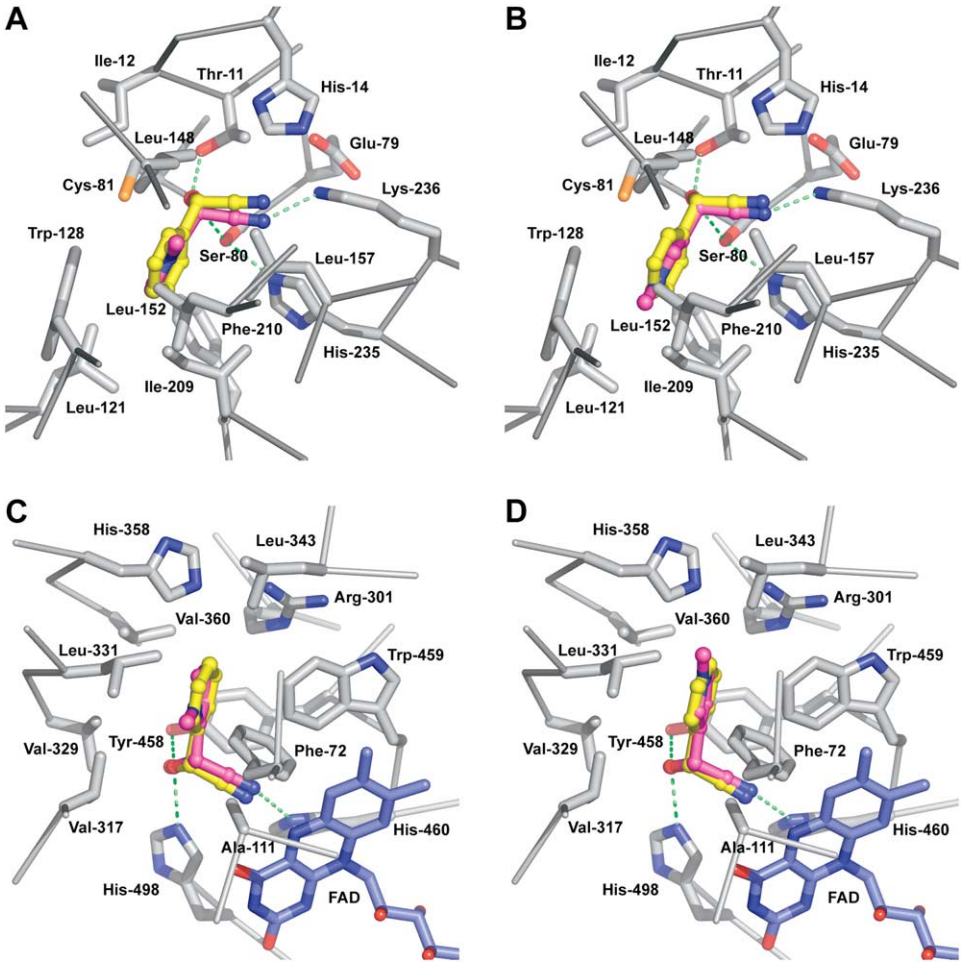


Figure 1. Modeled complexes of *Hb*HNL and *Pa*HNL5 with cyanohydrins from *N*-methyl pyrrole-carboxaldehydes in comparison with bound mandelonitrile: (A) complex of *Hb*HNL with unprotected *S*-**3a**, (B) complex of *Hb*HNL with unprotected *S*-**6a**, (C) complex of *Pa*HNL5 with unprotected *R*-**3a** and (D) complex of *Pa*HNL5 with unprotected *R*-**6a**. The pyrrole derivatives are shown in magenta, mandelonitrile is shown in yellow. Surrounding active site residues (within 5 Å) are shown in grey. Potential hydrogen bonding interactions are indicated by green dashed lines. Figures 1 and 2 were produced using the program PyMol (http://www.pymol.org/).

Thr-11. When the side chain of W128 (in its experimentally determined conformation)[7a] is reintroduced into the modeled complex of the W128A mutein and the benzylated substrate unprotected *S*-**7a**, severe steric clashes between the phenyl ring and the indole moiety of Trp-128 become evident (Fig. 2).

Thus, the most intriguing conclusion from these results is the indication of a pronounced flexibility of the bulky indole ring of Trp-128 in the wild type enzyme to allow larger carbonyl compounds—carrying hydrophobic groups—to be accommodated in the active site of *Hb*HNL. Thereby, the large substituent (e.g., benzyl) is bound in a predominantly hydrophobic region. Furthermore, this result serves as a likely explanation for the fact that pyrrole-2-carboxaldehydes carrying more polar substituents such as *tert*-butoxycarbonyl, benzyloxycarbonyl or tosyl on the nitrogen atom could not be converted to the desired cyanohydrins in a stereoselective manner in the presence of *Hb*HNL (data not given).

In the case of the phenyl substituted cyanohydrins, which lack the conformational flexibility provided by the

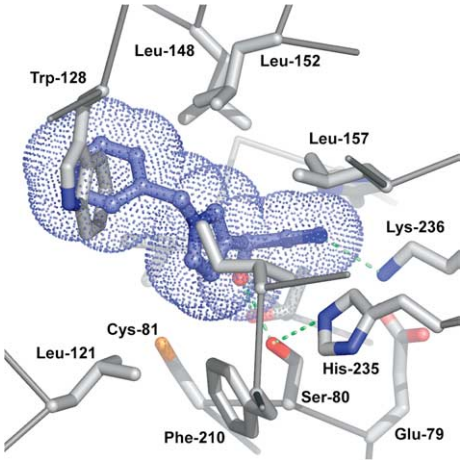


Figure 2. Modeled complex of *Hb*HNL with the benzylated compound unprotected *S*-**7a**. This complex was modeled based on a modified protein structure in which Trp-128 was replaced by alanine. In this figure, however, Trp-128 is shown in the conformation which was observed in the crystal structure.[7a]

methylene group present in the benzyl derivatives, suitable complexes were not found even for the W128A mutein. Visual inspection of possible, productive binding modes (preserving the mechanistically important polar interactions with Ser-80, Thr-11 and Lys-236) revealed severe clashes of the phenyl ring with surrounding amino acids. Experimental data support this prediction (Tables 1 and 2). However, it remains unclear why racemic **5a** could be isolated after silylation whereas **8** did not react at all.

In the case of *Pa*HNL5, it was not possible to identify one single amino acid residue playing a comparable role for the binding of structurally demanding substrates as indicated for Trp-128 in *Hb*HNL. Nevertheless, similar conformational flexibility can be assumed to be responsible for the appropriate binding of benzylated aldehydes **4** and **7** since these substrates have been successfully converted to the corresponding enantiomerically enriched cyanohydrins **4a** and **7a** in the presence of *Pa*HNL5 (Table 2).

The complementary stereopreference of the two enzymes was sustained for the investigated substrates. According to chiral analyses, the dominating enantiomers obtained with *Hb*HNL and *Pa*HNL5 had opposite absolute configurations in all cases.

3. Conclusion

Comparing the two enzymes, it can be concluded that upon *N*-alkylation pyrrole-carboxaldehydes can be transferred into the corresponding enantiomerically enriched cyanohydrins (at least **3**, **4**, **6** and **7**) in the presence of hydroxynitrile lyases from *H. brasiliensis* and *P. amygdalus* although with low to moderate yields and mostly moderate enantioselectivities. The enormously slow reaction rates seem to be due to the poor reactivity of the carbonyl group within this class of compounds (Table 3). Modeling calculations indicate the necessity of considerable flexibility of Trp-128 in the *Hevea* enzyme in order to adequately accommodate bulkier substrates. These predictions are consistent with the experimental results.

4. Experimental

4.1. Materials and methods

All solvents and materials not described in this chapter were commercially available and appropriately purified, if necessary. ^{1}H and ^{13}C NMR spectra were recorded on a Varian GEMINI 200 (^{1}H 199.92 MHz, ^{13}C 50.25 MHz). HPLC analyses were performed using a CHIRALCEL OD-H column (from DAICEL) on an Agilent 1100 Series instrument equipped with a G1365B MWD UV detector (254 nm). HPLC solvents were purchased from Merck. GC analyses were carried out on a Hewlett Packard 6890 instrument equipped with a Chirasil-DEX CB column and a FID. Optical rotation was measured on a Perkin Elmer 341 polarimeter. Mass spectra (EI, 70 eV) were recorded on a KRATOS profile HV-4 double focussing magnetic sector instrument. TLC was performed on silica gel 60 F_{254} aluminium plates (Merck), mixtures of cyclohexane and EtOAc were used as eluent and compounds were detected with UV (254 nm) and spraying with Mo-reagent (10% H_2SO_4, 10% $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, and 0.8% $Ce(SO_4)_2\cdot 4H_2O$ in water).

4.2. Docking calculations

Models for both enantiomers of the cyanohydrins **3a** to **8a** were built and optimized using the program Sybyl v6.5 (Tripos Inc.). Partial atomic charges for these compounds were calculated using the RESP protocol,[16] parameters for the oxidized FAD cofactor were kindly provided by Wohlfahrt.[17] For the hydroxynitrile lyase from *H. brasiliensis* (*Hb*HNL), protein coordinates were taken from the respective atomic resolution X-ray crystal structure (PDB-entry: 1qj4).[18] For the enzyme from *P. amygdalus* (*Pa*HNL5), a homology model of isoenzyme #5 was used as in a previous modeling study.[12] This model is based on the crystal structure of isoenzyme #1 (*Pa*HNL1, PDB-entry: 1ju2)[7b], which shares about 75% sequence identity. In both protein models, Asp-, Glu-, Arg- and Lys-residues were treated as charged. Protonation and tautomerization states of His-residues were chosen that resulted in sensible hydrogen bonding networks. Hydrogen atoms were added to the structure, followed by a geometry optimization using AMBER 6.0[19] applying harmonic restraints on the positions of all heavy atoms. Only polar hydrogen atoms of the protein and the ligands were retained for the docking simulations.

The cyanohydrins were docked to these sites with the program AutoDock v3.0[20] restricting the search to a 22.5 Å cube. In all calculations employing a genetic algorithm optimization, the proteins were kept rigid, while the position and orientation of the ligands as torsion angles around single bonds were allowed to vary. Twenty five independent simulations with populations consisting of 50 random structures evolving in about 90–100 generations were performed. The best individual of each generation automatically survived. The probability for performing a local search (consisting up to 300 iterations) of a pseudo Solis & Wets optimization[21] was 10%. The lowest energy structures of each independent run were clustered using an rms-tolerance of 1.5 Å.

4.3. Quantum-chemical calculations

Structures of aldehydes and the corresponding cyanide adducts were fully optimized at the B3LYP/6-31+g(d) level of theory using Gaussian03.[22] Solvation energies for an aqueous solution were estimated using the program Jaguar v4.2 (Schrodinger Inc.). Relative reactivities (compared to benzaldehyde) of aldehydes towards the addition of cyanide were estimated as the zero-point and solvation corrected reaction energies of the isodesmic reaction:

$$R-CHO + Ph-CH(O^-)-CN \rightarrow R-CH(O^-)-CN + Ph-CHO$$

4.4. Syntheses

4.4.1. *N*-Triisopropylsilylpyrrole (1).[8b] To a stirred

solution of freshly distilled diisopropylamine (36.7 mL, 260 mmol) in 40 mL anhydrous THF was added a solution of *n*-butyllithium (2.5 M in hexane, 104 mL, 260 mmol) at −80 °C under an argon atmosphere. The solution was allowed to warm to room temperature. After rapid cooling to −80 °C pyrrole (18.0 mL, 259 mmol) was added and the solution was allowed to warm to room temperature again. After rapid cooling to −80 °C triisopropylsilylcloride (50.0 mL, 236 mmol) was added. After stirring for additional 15 h the solvent volume was reduced to 150 mL and the reaction mixture was partitioned between sat. $NaHCO_3$ (200 mL) and dichloromethane (200 mL). The organic layer was dried over Na_2SO_4 and removed under reduced pressure. Distillation of the residue afforded 48.9 g of **1** (93%) as a yellow liquid, bp 87–89 °C/1.2 mbar. ^{1}H NMR (200 MHz, $CDCl_3$): δ=1.12 (d, J=7 Hz, 18H), 1.48 (sept, J=7 Hz, 3H), 6.34 (t, 2H), 6.82 (t, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=11.9, 18.0, 110.2, 124.2 ppm. HRMS (EI): *m*/*z* calcd for $C_{13}H_{25}NSi$: 223.1756, found: 223.1760.

4.4.2. Pyrrole-3-carboxaldehyde (2b).[8] A solution of DMF (0.8 mL, 10.33 mmol) in 5 mL of dry dichloromethane was added dropwise to a stirred solution of oxalyl chloride (1.0 mL, 10.52 mmol) in 60 mL of dry dichloromethane at 0 °C. The white suspension was stirred for 30 min at 0 °C and then a solution of *N*-triisopropylsilylpyrrole (2.0 mL, 8.09 mmol) in 7 mL of dry dichloromethane was added rapidly. Subsequently the reaction mixture was heated to reflux for 30 min. The solvent was removed under reduced pressure almost quantitatively and the residue was stirred in a 5% aqueous sodium hydroxide solution at room temperature for 20 h. The solution was exhaustively extracted with dichloromethane in a continuous fashion. The organic layer was dried over Na_2SO_4 and removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 3:1) yielded 0.52 g of **2b** (67%) as an orange oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=6.67 (n.r., 1H), 6.85 (n.r., 1H), 7.48 (n.r., 1H), 9.80 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=107.6, 121.2, 126.8, 128.2, 186.7 ppm. HRMS (EI): *m*/*z* calcd for C_5H_5NO: 95.0371, found: 95.0350.

4.4.3. *N*-Benzylpyrrole-2-carboxaldehyde (4).[10] To a stirred solution of pyrrole-2-carboxaldehyde (3.1 g, 32.5 mmol) and tetrabutylammonium bromide (1.05 g, 3.25 mmol) in 30 mL of dichloromethane were added benzylbromide (4.0 mL, 37.2 mmol) at once and aqueous sodium hydroxide (9.0 g, 225 mmol in 18 mL of water) dropwise over a period of 0.5 h at 0 °C. Subsequently the reaction mixture was heated to reflux for 1 h. After stirring for additional 15 h water (25 mL) and dichloromethane (50 mL) were added. The organic phase was washed with 2 M HCl (25 mL), sat. $NaHCO_3$ (25 mL) and water (25 mL) and removed under reduced pressure. Distillation of the residue afforded 5.3 g of **4** (87%) as a brown oil, bp 123–125 °C/1.4 mbar. ^{1}H NMR (200 MHz, $CDCl_3$): δ=5.58 (s, 2H), 6.29 (t, J=3 Hz, 1H), 6.99 (n.r., 2H), 7.17 (n.r., 2H), 7.30 (m, 3H), 9.57 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=52.2, 110.4, 125.1, 127.5, 128.0, 129.0, 131.7, 131.8, 137.8, 179.8 ppm. HRMS (EI): *m*/*z* calcd for $C_{12}H_{11}NO$: 185.0841, found: 185.0833.

4.4.4. *N*-Phenylpyrrole-2-carboxaldehyde (5). Pyrrole-2-carboxaldehyde (2.00 g, 21.03 mmol), phenylboronic acid (5.56 g, 45.60 mmol), copper (II) acetate (5.98 g, 32.92 mmol) and triethylamine (6.0 mL, 43.11 mmol) were stirred in 20 mL of anhydrous dichloromethane at room temperature for 18 h. The reaction mixture was filtered through Celite®, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 7:1) yielded 2.93 g of **5** (81%) as an orange oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=6.37 (n.r., 1H), 7.04 (n.r., 1H), 7.14 (dd, 1H), 7.29–7.46 (m, 5H), 9.52 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=111.2, 122.4, 126.3, 128.5, 129.4, 131.4, 132.8, 139.0, 179.4 ppm. HRMS (EI): *m*/*z* calcd for $C_{11}H_9NO$: 171.0684, found: 171.0685.

4.4.5. *N*-Methylpyrrole-3-carboxaldehyde (6).[9] To a stirred solution of pyrrole-3-carboxaldehyde (1.30 g, 13.67 mmol) in 30 mL of cyclohexane/DMSO (2:1) was added potassium *tert*-butoxide (1.93 g, 17.20 mmol) and 18-crown-6 (440 mg, 1.66 mmol). Subsequently iodomethane (2.5 mL, 40.16 mmol) was added dropwise over a period of 10 min. After completion of the reaction (GC) water was added (35 mL) and the mixture was extracted with ethyl acetate (4×40 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 2:1) yielded 1.27 g of **6** (85%) as a light brown liquid. ^{1}H NMR (200 MHz, $CDCl_3$): δ=3.70 (s, 3H), 6.60 (n.r., 2H), 7.23 (n.r., 1H), 9.70 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=37.0, 108.7, 124.6, 126.9, 130.1, 185.5 ppm. HRMS (EI): *m*/*z* calcd for C_6H_7NO: 109.0528, found: 109.0522.

4.4.6. *N*-Benzylpyrrole-3-carboxaldehyde (7).[10] To a stirred solution of pyrrole-3-carboxaldehyde (1.00 g, 10.52 mmol) in 10 mL of dichloromethane was added tetra-*n*-butylammonium bromide (337 mg, 1.05 mmol) and benzyl bromide (1.50 mL, 12.63 mmol). Subsequently aqueous sodium hydroxide (4.2 g, 105 mmol in 10 mL of water) was added dropwise at 0 °C. After completion of the addition, the solution was allowed to warm to room temperature. After 5 h 50 mL of a 10% HCl solution were added slowly and the phases were separated. The organic phase was washed with sat. $NaHCO_3$, dried over Na_2SO_4 and removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 3:1) yielded 1.73 g of **7** (88%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=5.09 (s, 2H), 6.68 (m, 2H), 7.17 (m, 2H), 7.30–7.37 (m, 4H), 9.73 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=54.2, 108.9, 124.0, 127.1, 127.6, 128.6, 129.3, 129.4, 136.4, 185.6 ppm. HRMS (EI): *m*/*z* calcd for $C_{12}H_{11}NO$: 185.0841, found: 185.0842.

4.4.7. *N*-Phenylpyrrole-3-carboxaldehyde (8). Pyrrole-3-carboxaldehyde (1.50 g, 15.77 mmol), phenylboronic acid (3.85 g, 31.54 mmol), copper(II)acetate (4.30 g, 23.66 mmol) and triethylamine (4.6 mL, 33.12 mmol) were stirred in 40 mL of anhydrous dichloromethane at room temperature for 72 h. The reaction mixture was filtered through Celite®, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by column chromatography (cyclohexane/EtOAc 7:1) yielded

1.16 g of **8** (43%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=6.81 (dd, J=3, 2 Hz, 1H), 7.09 (n.r., 1H), 7.36–7.50 (m, 5H), 7.67 (n.r., 1H), 9.86 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=109.9, 121.4, 122.6, 127.4, 127.6, 128.4, 130.1, 139.8, 185.7 ppm. HRMS (EI): *m/z* calcd for $C_{11}H_9NO$: 171.0678, found: 171.0684.

4.4.8. Synthesis and safe-handling of anhydrous HCN—CAUTION. All reactions involving HCN or cyanides were carried out in a well ventilated hood. For continuous warning, an electrochemical sensor for HCN detection was used. The required amount of HCN was freshly prepared by dropping a saturated NaCN solution into aqueous sulfuric acid (60%) at 80 °C. HCN was transferred through a drying column in a nitrogen stream and collected in a cooling trap at −12 °C. Waste solutions containing cyanides were treated with aqueous sodium hypochlorite (10%). Subsequently the pH was adjusted to 7.0 with aqueous sulfuric acid.

4.4.9. General procedure for the synthesis of racemic cyanohydrins. *Procedure A*. To a stirred solution of the corresponding aldehyde in acetonitrile were added TBDMSCl (1.5 equiv), KCN (4 equiv) and a catalytic portion of ZnI_2. The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction the mixture was partitioned between dichloromethane and a saturated solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure.

Procedure B. To a stirred solution of the corresponding aldehyde in *tert*-butyl methyl ether in the presence of Amberlyst-A21 was added HCN (10 equiv) via a syringe. The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction, the solvent and HCN were removed under reduced pressure. Subsequently the crude cyanohydrins were silylated (see Section 4.4.11).

4.4.10. General procedure for the enzymatic cyanohydrin reaction. To a stirred solution of aldehyde in *tert*-butyl methyl ether was added a buffered (sodium citrate 100 mM) solution of the corresponding hydroxynitrile lyase (*Hb*HNL pH=4.8, *Pa*HNL5 pH=3.5–4.0). After 10 min HCN (5 or 10 equiv, see Tables 1 and 2) was added and the mixture was stirred at 0 °C for approximately 2 h and subsequently allowed to warm to room temperature. For work-up Celite® was added and the mixture was transferred into a filter funnel containing Na_2SO_4. The product and remaining starting material were washed out with *tert*-butyl methyl ether. The collected solvent was removed under reduced pressure and the crude cyanohydrins were silylated (see Section 4.4.11).

4.4.11. General procedure for silylation of crude cyanohydrins. To a stirred solution of crude cyanohydrin in DMF was added imidazole (1.5 equiv) and TBDMSCl (1.2 equiv). The reaction was stirred at room temperature and monitored by TLC. After completion of the reaction dichloromethane was added and the mixture was extracted with HCl 10%, sat. $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and removed under reduced pressure.

4.4.12. *tert*-Butyl dimethylsilyloxy(*N*-methylpyrr-2-yl) acetonitrile (3a). Purification by column chromatography (cyclohexane/EtOAc 2:1) yielded **3a** as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.05 (s, 3H), 0.13 (s, 3H), 0.90 (s, 9H), 3.77 (s, 3H), 5.54 (s, 1H), 6.05 (t, J=Hz, 1H), 6.19 (dd, J=3, 2 Hz, 1H), 6.69 (t, J=2 Hz, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=−5.1, −5.0, 18.3, 25.7, 34.6, 58.0, 107.4, 110.7, 118.4, 125.7, 126.2 ppm. HRMS (EI): *m/z* calcd for $C_{13}H_{22}N_2OSi$: 250.1492, found: 250.1501. (chiral GC analysis: Chirasil-DEX, 110 °C, 1 bar H_2; ret. times: 11.4, 12.6 min).

4.4.13. *tert*-Butyl dimethylsilyloxy(*N*-benzylpyrr-2-yl) acetonitrile (4a). Purification by column chromatography (cyclohexane/EtOAc 20:1) yielded **4a** as a white solid (mp 35–37 °C). ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.08 (s, 6H), 0.87 (s, 9H), 5.20 (d, J=16 Hz, 1H), 5.31 (d, J=16 Hz, 1H), 5.50 (s, 1H), 6.14 (t, J=4 Hz, 1H), 6.33 (d, J=4, 2 Hz, 1H), 6.67 (t, J=2 Hz, 1H), 7.10 (m, 2H), 7.29–7.38 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=−5.1, −4.9, 18.3, 25.7, 51.1, 58.0, 108.0, 110.8, 118.5, 125.0, 126.7, 127.3, 128.0, 129.0, 137.5 ppm. HRMS (EI): *m/z* calcd for $C_{19}H_{26}N_2OSi$: 326.1827, found: 326.1814. (chiral HPLC analysis: CHIRALCEL OD-H, *n*-heptane/2-propanol 95:5, 0.5 mL/min, 10 °C; ret. times: 10.3, 13.6 min).

4.4.14. *tert*-Butyl dimethylsilyloxy(*N*-phenylpyrr-2-yl) acetonitrile (5a). Purification by column chromatography (cyclohexane/EtOAc 50:1) yielded **5a** as a dark yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.1 (s, 3H), 0.04 (s, 3H), 0.85 (s, 9H), 5.48 (s, 1H), 6.29 (t, J=3 Hz, 1H), 6.59 (dd, J=3, 2 Hz, 1H), 6.87 (t, J=2 Hz, 1H), 7.38–7.47 (m, 5H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=−5.0, −4.9, 18.2, 25.7, 57.2, 108.9, 112.1, 118.7, 125.2, 126.6, 127.9, 128.4, 129.6, 139.2 ppm. HRMS (EI): *m/z* calcd for $C_{18}H_{24}N_2OSi$: 312.1649, found: 312.1658. (chiral HPLC analysis: CHIRALCEL OD-H, *n*-heptane/2-propanol 99:1, 0.5 mL/min, 10 °C; ret. times: 9.9, 10.9 min).

4.4.15. *tert*-Butyl dimethylsilyloxy(*N*-methylpyrr-3-yl) acetonitrile (6a). Purification by column chromatography (cyclohexane/EtOAc 20:1) yielded **6a** as a light brown oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.15 (s, 3H), 0.18 (s, 3H), 0.93 (s, 9H), 3.64 (s, 3H), 5.48 (s, 1H), 6.18 (t, J=3, 2 Hz, 1H), 6.57 (t, J=3, 2 Hz, 1H), 6.73 (s, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=−4.8, −4.7, 18.4, 25.8, 36.6, 59.0, 107.3, 120.1, 120.4, 120.5, 122.9 ppm. HRMS (EI): *m/z* calcd for $C_{13}H_{22}N_2OSi$: 250.1490, found: 250.1501. (chiral HPLC analysis: CHIRALCEL OD-H, *n*-heptane/2-propanol 95:5, 0.5 mL/min, 10 °C; ret. times: 10.9, 11.9 min).

4.4.16. *tert*-Butyl dimethylsilyloxy(*N*-benzylpyrr-3-yl) acetonitrile (7a). Purification by column chromatography (cyclohexane/EtOAc 50:1) yielded **7a** as a pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.13 (s, 3H), 0.16 (s, 3H), 0.92 (s, 9H), 5.04 (s, 2H), 5.50 (s, 1H), 6.23 (t, J=3, 2 Hz, 1H), 6.65 (t, J=3, 2 Hz, 1H), 6.80 (s, 1H), 7.11–7.15 (m, 2H), 7.30–7.35 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=−4.8, −4.7, 18.4, 25.8, 53.8, 59.1, 107.7, 120.0, 120.1, 120.8, 122.4, 127.4, 128.2, 129.1, 137.6 ppm. $[\alpha]_D^{20}$ −6.7 (*c* 0.52, C_2H_5OH, 91% ee, (*S*)). HRMS (EI): *m/z* calcd for $C_{19}H_{26}N_2OSi$: 326.1811, found: 326.1814. (chiral HPLC

analysis: CHIRALCEL OD-H, *n*-heptane/2-propanol 95:5, 0.5 mL/min, 10 °C; ret. times: 16.3, 21.8 min).

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