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A study of asymmetric hydrocyanation of heteroaryl carboxaldehydes catalyzed by (*R*)-oxynitrilase under micro-aqueous conditions

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Abstract—A number of new optically active heteroaryl cyanohydrins have been prepared by hydrocyanation under micro-aqueous conditions catalyzed by almond meal (containing (*R*)-oxynitrilase). Substituent effects on the reaction are discussed. This micro-aqueous method provides an efficient, convenient and economical approach to optically active cyanohydrins. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Optically active cyanohydrins are useful intermediates in the synthesis of a variety of natural and non-natural compounds of biological interest because the stereogenic carbon atom in the molecule bears two functionalities, which can readily undergo further transformation. For example, the preparation of homochiral amino alcohols starting from optically active cyanohydrins in most cases is easier and more efficient. Among cyanohydrins, heteroaryl cyanohydrins are of special importance, which constitute the structural moiety of many biologically active molecules.[1] Routes to optically active cyanohydrins can be categorized into non-enzymatic and enzymatic approaches. In the former there are the chiral complex-controlled methods and the cyclic dipeptide-catalyzed method, while in the latter, transformations by lipases and oxynitrilases have been reported.[2] However, only a few chiral heteroaryl cyanohydrins containing *O*- or *S*-atoms in the heterocyclic ring have been successfully synthesized by the isolated or recombinant (*R*)- or (*S*)-oxynitrilases, whilst the hydrocyanations of *N*-containing heteroaryl carboxaldehydes have been reported to be unsuccessful.[2]

In analyzing the structural feature of some *NH*-containing heteroaryl carboxaldehydes, we hypothesised that it might be the hydrogen bond between the N–H and the aldehyde carbonyl group that causes the inhibition of the hydrocyanation reaction. Elimination of the hydrogen bond by protecting the NH group might therefore provide a way to improve the situation. In addition, we were also interested in undertaking an investigation into the effect of ring substituents on the hydrocyanation reaction. In many cases, almond meal has been proven to be as efficient as the purified enzyme and as convenient as the immobilized enzyme by Brussee[3] and by ourselves.[4] It is known that reaction in an organic solvent can effectively depress the non-stereoselective addition of HCN to the carbonyl group via chemical pathway and minimize the reversible equilibrium between HCN addition and cyanohydrin decomposition. As such, we carried out the hydrocyanation of a variety of substituted heteroaryl carboxaldehydes using almond meal as the catalyst under 'micro-aqueous conditions'.[4] Herein we wish to report our results.

2. Results and discussion

2.1. 'Micro-aqueous conditions' for the asymmetric hydrocyanation of heteroaryl carboxaldehydes

In efforts to optimise the reaction conditions with almond preparations as the catalyst, we found that dried almond powder gave no hydrocyanation product, even when the reaction was carried out in water-saturated *iso*-propyl ether (IPE) (which was determined to

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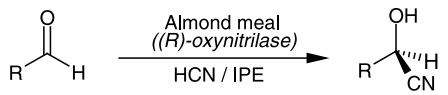
contain about 0.3% water). Therefore, almond kernels were firstly saturated with water then pulverized, which resulted in a water content of about 9% in the tissue of the almond powder. This water content satisfies the need of the enzyme for essential water to exhibit its full activity, and the organic medium used for the reaction has so little water content that it can effectively prevent the resultant cyanohydrins from decomposition into the starting aldehyde and racemization, additionally the reactions are carried out at ambient temperature. In order to compare the stability of the resulting cyanohydrin in aqueous buffer and in *iso*-propyl ether, (*S*)-2-(2-furanyl)-2-hydroxynitrile was dissolved in citrate buffer (pH 5.5) and in isopropyl ether, respectively. The formation of the 2-furanylcarboxaldehyde by decomposition and racemization of the cyanohydrin was determined for the two conditions. It was found that the cyanohydrin underwent rapid decomposition and racemization in aqueous solution, whereas it was fairly stable in *iso*-propyl ether. Therefore, micro-aqueous conditions[4] can provide an environment for the hydrocyanation reaction which minimizes the decomposition and racemization of the formed cyanohydrins.

The general hydrocyanation reaction is shown in the following scheme.



R = heteroaryl ring

2.2. Furanyl carboxaldehydes

Transformation of furan-2-yl and 5-methylfuran-2-yl carboxaldehyde to the corresponding cyanohydrins has been accomplished under the catalysis of (*R*)-oxynitrilase isolated from almond nuts (*Prunus amygdalus*) with an e.e. value of 99% and yield of 96[5] or 88%[6a] in the former case and with an e.e. value of 99% and yield of 70% in the latter case.[7a] The results obtained by performing the reaction under micro-aqueous conditions with these two substrates are similar to those reported previously (Table 1, entry a, b). Methyl substitution at the 5-position leads to a significant decrease in the yield, which may be attributed to steric hindrance resulting in poor fit between the substrate and the enzyme. 2-Methyl-furanyl-3-carboxaldehyde (entry c) gave moderate yield and low e.e. The reaction was totally inhibited when the furan ring bore a hydroxymethyl or acetoxymethyl group at the 5-position. Introduction of a nitro group caused the aldehyde to be so reactive that a tar formed without any desired product detected.

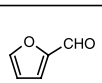
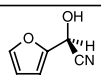
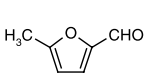
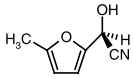
2.3. Thien-2-yl carboxaldehydes

Thien-2-yl cyanohydrin was obtained under our conditions in similar yield and stereoselectivity to those reported by Effenberger et al.[5] (Table 2, entry a). Introduction of a methyl substituent at the 3-position of the thienyl ring also resulted in a significant decrease both in the yield and enantiomeric excess as in the case of 5-methylfuran-2-yl cyanohydrin mentioned above (entry c). Interestingly, bromo substitution at the 5-position maintained the yield of the cyanohydrin at the same level as the non-substituted substrate and did not decrease the product e.e. as much as in the case of methyl substitution (entry b). This may be a result of a balance between the activation effect on the carbonyl group and steric hindrance from the bromo moiety.

2.4. Pyrrolylcarboxaldehydes and indolylcarboxaldehydes

The use of *N*-containing five-membered heteroaryl carboxaldehydes as the substrate of enzymatic hydrocyanation has so far not been extensively studied. As reported by Griengl et al.,[8] pyrrolyl- and indolyl-car-

Table 1. Hydrocyanation of some furanyl carboxaldehydes under micro-aqueous conditions

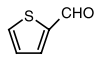
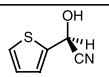
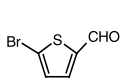
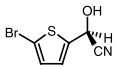
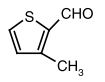
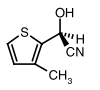
Entry	Substrate	Product	Yield/e.e. (%)	Configuration
a		 **1a**	100/99	$S^{*,a}$
b		 **1b**	60/97	$S^{*,b}$
c		 **1c****	78/24.3	$S^{*,c}$

*The absolute configurations were assigned in the light of stereochemical preference of the enzyme, (*R*)-oxynitrilase. The (*S*)-configurations are due to the Cahn-Ingold-Prelog rules.

**Isolated as its benzoate.

[a.] Retention time: 9.14min for **1a** and 10.55min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 9:1 flow rate: 0.7mL/min, monitored at 254nm); [b.] 19.39min for **1b** and 24.60min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 100:1.25, flow rate: 0.7mL/min, monitored at 254nm); [c.] 10.32min for **1c** and 11.25min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm).

Table 2. Hydrocyanation of some thien-2-yl carboxaldehydes under micro-aqueous conditions

Entry	Substrate	Product	Yield/e.e. (%)	Configuration
a		 **2a**	70/99	$S^{*,a}$
b		 **2b**	72/86	$S^{*,b}$
c		 **2c**	50/65	$S^{*,c}$

*The absolute configurations were assigned in the light of stereochemical preference of the enzyme, (*R*)-oxynitrilase. The (*S*)-configurations are due to the Cahn-Ingold-Prelog rules.

[a.] Retention time: 9.58min for **2a** and 10.54min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1 flow rate: 0.7mL/min, monitored at 254nm); [b.] 16.15min for **2b** and 18.20min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm); [c.] 13.12min for **2c** and 14.23min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm).

boxaldehydes gave no product in the hydrocyanationreaction catalyzed either by isolated (*R*)-oxynitrilase (from almond, *Prunus amygdalus*) or by (*S*)-oxynitrilase (from rubber tree, *Hevea brasiliensis*). We assumed that the intra- or intermolecular hydrogen bonds between the hydrogen atom of the ring nitrogen and the oxygen atom of the carbonyl group in the substrate molecules might deactivate the carbonyl group. Therefore, a series of *N*-blocked substrates was tested for hydrocyanation under our conditions. The results appear to support our assumption. Most of the *N*-blocked substrates gave the corresponding cyanohydrins with varying yields and enantiomeric excesses. Although many substrates did not give products with preparative significance, the results in some cases are promising and practical. *N*-Ts and *N*-Boc protected pyrrolylcarboxaldehydes (entry a and b) gave no good results (both yield and e.e.), probably due to the bulk of the protecting groups, which may interfere with the interaction between the substrate and the enzyme. Pyrrolylcarboxaldehydes with *N*-blocked by a methyl (Table 3, entry d) or a methoxymethyl group (MOM) (entry c) afforded the corresponding cyanohydrins in low yields and with modest enantioselectivities. Substitution at the 5-position with an acetyl (entry e) or a cyano group (entry f) significantly improved the yields with moderate enantiomeric excesses. Nitro- or chloro-substituents at position 5 gave adverse effects, resulting in the formation of a tar. A methoxy group on the 5-position afforded no reaction with recovery of the starting material. In the case of the indolyl series, compared to the parent compound, blocking the nitrogen atom by Boc promoted the reaction (entry g), although the result was still not satisfactory. This may be due to the bulky backbone of the aryl ring itself, which results in poor fit with the enzyme. Protonation of the nitrogen atom under the acidic medium may be the other reason which results in an adverse effect on the outcome of the reaction.

2.5. Pyridinyl-carboxaldehydes

All pyridinyl 2-, 3- and 4-carboxaldehydes have been reported to give no hydrocyanation product under the catalysis of (*S*)-oxynitrilase[8b] isolated from rubber tree (*Hevea brasiliensis*). In the case of the enzyme isolated from almond (*Prunus amygdalus*), pyridinyl 3-carboxaldehyde was reported to give (*R*)-pyridin-3-yl cyanohydrin in 89% yield and 14% e.e.,[6a] and 97% yield and 82% e.e.,[6b] respectively, whereas the transformation of other pyridinyl carboxaldehydes has not been reported.

Under our reaction conditions, the results of the hydrocyanation of pyridinyl-carboxaldehydes varied dramatically with the position of the aldehyde group on the pyridine ring (Table 4). Pyridin-2-yl carboxaldehyde (entry a) gave the corresponding cyanohydrin in moderate yields and 0 e.e., pyridin-3-yl carboxaldehyde (entry d) produced its cyanohydrin in 99.5% yield and 50% e.e. and pyridin-4-yl carboxaldehyde formed a tar. Substitution on the pyridine ring also gave varied results. Again, bromo substitution at a position *ortho* to the hetero-atom improved both the yield and e.e. (entry c), whereas methyl substitution at the same position provided no good effect (entry b).

2.6. Heteroaryl-carboxaldehydes containing two heteroatoms in the ring

The enzymatic hydrocyanation of heteroaryl carboxaldehydes containing two heteroatoms in the ring has so far not been reported. 1-*N*-Methyl-imidazol-2-yl carboxaldehyde gave the corresponding cynaohydrin in high yield and low e.e. (Table 5, entry a), but no hydrocyanation reaction occurred with both unprotected imidazolyl and 1-*N*-MOM-imidazol-2-yl carboxaldehydes. Fortunately, thiazol-2-yl cyanohydrin was obtained from the carboxaldehyde in good yield and

moderate enantiomeric excess (Table 5, entry b), which we believe will find appreciable applications in the synthesis of biologically interesting compounds.

3. Conclusions

A number of new optically active heteroaryl cyanohydrins have been prepared from heteroaryl carboxaldehydes under the catalysis of almond meal using micro-aqueous conditions.[4] The effect of substituents of the heteroaryl ring on the reaction has also been examined. Introduction of a substituent generally gave results in both yield and e.e., which were no better than the corresponding parent compounds. It seems that electron-donating groups such as methyl, hydroxymethyl, and methoxy groups reduce the reactivity of the aldehydes preventing the hydrocyanation reaction from occurring; the presence of strong electron-withdrawing groups such as a nitro group activates the aldehyde so much that a black tar was formed without any desired products detected; heteroaryl carboxaldehydes substituted with moderate electron-withdrawing groups such as halogen and cyano groups usually gave modest yields and e.e.s.

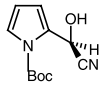
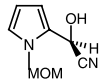
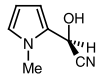
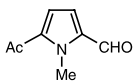
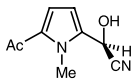
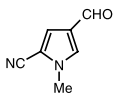
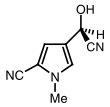
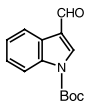
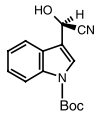
It is believed that these new heteroaryl cyanohydrins will find application in the synthesis of biologically interesting molecules.

4. Experimental

4.1. Materials and methods

All of the heteroaryl carboxaldehydes used were purchased from Acros Organics. *N*-Protection with Boc, methoxymethyl, methyl, and tosyl groups for pyrrolyl-, indolyl- and imidazolyl-carboxaldehydes was performed by known methods.

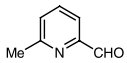
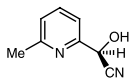
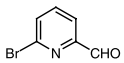
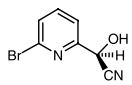
Table 3. Hydrocyanation of some pyrrolyl- and indolylcarboxaldehydes under micro-aqueous conditions

Entry	Substrate	Product	Yield/e.e. (%)	Confoguration
a		 **3a**	4.2/0.9	$R^{*,a}$
b		 **3b**	4.5/2.2	$R^{*,b}$
c		 **3c**	33/81	$R^{*,c}$
d		 **3d**	17/40	$R^{*,d}$
e		 **3e**	99/34	$R^{*,e}$
f		 **3f**	84/67	$R^{*,f}$
g		 **3g**	2/8	$R^{*,g}$

*The absolute configurations were assigned in the light of stereochemical preference of the enzyme, (*R*)-oxynitrilase.

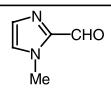
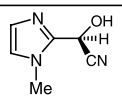
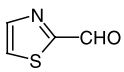
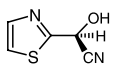
[a.] Retention time: 29.19min for **3a** and 40.16min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1 flow rate: 0.7mL/min, monitored at 254nm); [b.] 11.28min for **3b** and 12.38min for its enantiomer (Chiralpak AS column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm); [c.] 12.19min for **3c** and 13.30min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm); [d.] 10.57min for **3d** and 11.79min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 9:1 flow rate: 0.7mL/min, monitored at 254nm); [e.] 14.16min for **3e** and 14.76min for its enantiomer (Chiralpak OJ column, eluent: hexane/EtOH 3:7, flow rate: 0.7mL/min, monitored at 254nm); [f.] 21.14min for **3f** and 24.15min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 8:2, flow rate: 0.7mL/min, monitored at 254nm); [g.] 19.19min for **3g** and 24.58min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm).

Table 4. Hydrocyanation of some pyridinyl-carboxaldehydes under micro-aqueous conditions

Entry	Substrate	Product	Yield/e.e. (%)	Configuration
a		 **4a**	42/0	—[*,a]
b		 **4b**	38/0	—[*,b]
c		 **4c**	92/65	$R^{*,c}$
d		 **4d**	99.5/50	$R^{*,d}$

*The absolute configurations were assigned in the light of the stereochemical preference of the enzyme, (*R*)-oxynitrilase.
[a.] Retention time: 10.11min and 12.21min (Chiralpak AS column, eluent: hexane/2-PrOH 9:1 flow rate: 0.7mL/min, monitored at 254nm); [b.] 17.03min and 20.38min (Chiralpak AS column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm); [c.] 6.95min for **4c** and 7.58min for its enantiomer (Chiralpak OJ column, eluent: hexane/2-PrOH 9:1, flow rate: 0.7mL/min, monitored at 254nm); [d.] 14.55min for **4d** and 16.19min for its enantiomer (Chiralpak AD column, eluent: hexane/2-PrOH 8:2 flow rate: 0.7mL/min, monitored at 254nm).

Table 5. Hydrocyanation of some heteroaryl-carboxaldehydes containing two heteroatoms in the ring under micro-aqueous conditions

Entry	Substrate	Product	Yield/e.e. (%)	Configuration
a		 **5a**	94/5	$S^{*,a}$
b		 **5b**	97/67	$S^{*,b}$

*The absolute configurations were assigned in the light of stereochemical preference of the enzyme, (*R*)-oxynitrilase. The *S* configurations are due to the Cahn-Ingold-Prelog rules.
[a.] Retention time: 17.98min for **5a** and 18.71min for its enantiomer (Chiralpak OC column, eluent: hexane/2-PrOH 8:2 flow rate: 0.7mL/min, monitored at 254nm); [b.] 15.53min for **5b** and 17.08min for its enantiomer (Chiralpak OC column, eluent: hexane/2-PrOH 8:2, flow rate: 0.7mL/min, monitored at 254nm).

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM 300 (300 MHz) with TMS as an internal reference; EI mass spectra were measured on an HP 5989A; high resolution mass spectra (FAB) were measured on a Finnigan MAT 8430; infrared spectra were acquired on a Perkin Elmer 983 or Digibal FTIR; optical rotations were determined on a Perkin–Elmer 241MC. Enantiomeric excess values were obtained by first either acetylating or benzoylating the cyanohydrins by known methods, then subjecting the acylated products to HPLC analysis on Chiralpak columns. The e.e. determinations and elemental analyses on a Heraeus Rapid-CHNO elemental analyzer were performed by the Analytical Department of this Institute. Water contents in the crude enzyme and in *iso*-propyl ether used in the enzymatic reaction was determined on a Mettler DL35 Karl–Fischer titrator. The almond kernals used in this study were obtained from fresh fruits of almond produced in Lishi County, Shanxi Province.

4.2. Preparation of racemic cyanohydrins

A mixture of aldehyde (1 mmol), 10% aqueous $NaHSO_3$ (1.5 mL) and ethyl ether was stirred at 0°C for 1 h. To the stirred mixture 20% aqueous KCN (0.4 mL) was added. The resultant mixture was stirred at 0–30°C for 2–24 h. The aqueous phase was separated and extracted with ethyl ether (2×10 mL). The combined organic phase was washed with water (10 mL) and dried over Na_2SO_4. Flash chromatography on silica gel (eluent: ethyl acetate/petroleum ether) gave the corresponding racemic cyanohydrins, which were used as references in e.e. value determination.

4.3. Crude enzyme preparation

Almond kernels were swollen by soaking in distilled water for 2 h, peeled, air-dried and then pulverized in cooled ethyl acetate with a homogenizer. The resultant powder was filtered and de-fatted by three washes with ethyl acetate, filtered and stored in a refrigerator.

4.4. General procedure for enzymatic hydrocyanation

A mixture of aldehyde (1.25 mmol), almond meal (0.5 g), and 2 M HCN in isopropyl ether (5 mL) was stirred at 4–30°C for 16–100 h. Flash chromatography on silica gel (eluent: ethyl acetate/petroleum ether) provided the corresponding cyanohydrins.

4.4.1. (*S*)-(+)-2-Hydroxy-2-(2-furyl)acetonitrile 1a. Clear oil; yield: 100%; $[\alpha]_D^{21}$ +50.0 ($c=1.60$, $CHCl_3$), (lit[9] value: +50.3); e.e. 99%; ^{1}H NMR ($CDCl_3$): δ, 3.17 (br, s, 1H, OH), 5.55 (s, 1H, CH), 6.43 (dd, 1H, $J_1=1.9$ Hz, $J_2=3$ Hz, Ar-H), 6.61 (m, 1H, Ar-H), 7.49 (m, 1H, Ar-H).

4.4.2. (*S*)-(+)-2-Hydroxy-2-(5-methyl-2-furyl)acetonitrile 1b. Clear oil; yield: 60%; $[\alpha]_D^{21}$ +61.0 ($c=1.60$, $CHCl_3$); e.e. 97.2%; ^{1}H NMR ($CDCl_3$): δ, 2.5 (s, 3H, CH_3), 3.0 (s, 1H, OH), 5.5 (s, 1H, CH), 6.0–7.2 (m, 2H, Ar-H).

4.4.3. (*R*)-(+)-2-Benzoyloxy-2-(2-methyl-3-furanyl)acetonitrile 1c. Clear oil; yield 78%; $[\alpha]_D^{21}$ +8.2 ($c=6.5$, $CHCl_3$); e.e. 24.3%; ^{1}H NMR ($CDCl_3$): δ, 2.6 (s, 3H, CH_3), 5.5 (s, 1H, CH), 6.5–7.5 (m, 7H, Ar-H); IR (cm^{-1}): 3401, 2806, 2511, 1790, 1726, 1601, 1591, 1492, 1452, 1280, 1011, 996, 930, 869. MS: m/z (rel. intensity, %): 137 (M^+, 94), 120 (M^+–OH, 86), 119 (M^+–H_2O, 64), 110 (94), 109 (100), 81 (44), 53 (78), 43 (98); HRMS (FAB): $C_{14}H_{11}NO_3$. Found: 241.0799, calcd: 241.0740.

4.4.4. (*S*)-(+)-2-Hydroxy-2-(2-thienyl)acetonitrile 2a. Clear oil; yield: 70%; $[\alpha]_D^{21}$ +61.6 ($c=1.60$, $CHCl_3$); e.e. 99%; ^{1}H NMR ($CDCl_3$): δ, 3.0 (s, 1H, OH), 5.8 (s, 1H, CH), 6.9–7.4 (m, 3H, Ar-H).

4.4.5. (*S*)-(+)-2-Hydroxy-2-(5-bromo-2-thienyl)acetonitrile 2b. Clear oil; yield: 72%; $[\alpha]_D^{21}$ +19.7 ($c=1.60$, $CHCl_3$); e.e. 86%; ^{1}H NMR ($CDCl_3$): δ, 3.0 (s, 1H, OH), 5.7 (s, 1H, CH), 7.3–7.4 (m, 2H, Ar-H); IR (cm^{-1}): 3386, 3109, 2922, 1526, 1401, 1339, 1301, 1253, 1187, 1145, 1125, 1062, 1030, 903, 841, 820, 750, 628, 578; MS: m/z (rel. intensity, %): 219 (M^++H, 53) 217 (M^+–H, 54), 192 (M^+–CN, 67), 191 (M^+–HCN, 100), 190 (M^+–HCN–H, 86), 189 (91), 138 (67), 82 (45); HRMS (FAB): C_6H_4BrNOS. Found: 190.9183, calcd: 190.9166.

4.4.6. (*S*)-(+)-2-Hydroxy-2-(3-methyl-2-thienyl)acetonitrile 2c. Clear oil; yield: 48%; $[\alpha]_D^{21}$ +24.5 ($c=5.0$, $CHCl_3$); e.e. 65%; ^{1}H NMR ($CDCl_3$): δ, 2.5 (s, 3H, CH_3), 3.0 (s, 1H, OH), 5.8 (s, 1H, CH), 6.9–7.4 (m, 3H, Ar-H); IR (cm^{-1}): 3404, 3110, 2926, 2864, 2250, 1639, 1554, 1428, 1387, 1257, 1179, 1015, 942, 920, 846, 836, 728, 680, 589; MS: m/z (rel. intensity, %): 153 (M^+, 100), 136 (20), 126 (M^+–HCN, 88), 125 (M^+–HCN–H, 100), 97 (36), 53 (18), 45 (20), 44 (30); HRMS (FAB): C_7H_7NOS. Found: 153.0213, calcd: 153.0248.

4.4.7. (*R*)-(+)-2-Hydroxy-2-(2-(*N*-methoxymethyl)pyrrolyl)acetonitrile 3c. Clear oil; yield: 33%; $[\alpha]_D^{21}$ +104.9 ($c=0.667$, $CHCl_3$); e.e. 81%; ^{1}H NMR ($CDCl_3$): δ, 3.30 (s, 3H, CH_3), 3.92 (br, s, 1H, OH), 5.08–5.68 (AB, 2H, $J=10.9$ Hz, CH_2), 5.56 (s, 1H, CH), 6.13 (dd, 1H, $J_1=3.5$ Hz, $J_2=3.0$ Hz, Pyrr-H), 6.47 (dd, 1H, $J_1=3.6$ Hz, $J_2=1.7$ Hz, Pyrr-H), 6.83 (dd, 1H, $J_1=2.9$ Hz, $J_2=1.7$ Hz, Pyrr-H); ^{13}C NMR: δ, 126.38 (t-C-Pyrr), 125.59 (C-Pyrr), 118.01 (CN), 113.18 (C-Pyrr), 108.01 (C-Pyrr), 78.63 (NCH_2O), 56.55 (CHOH), 55.995 (OCH_3); IR (cm^{-1}): 3402, 3117, 2934, 2851, 2247, 1486, 1464, 1444, 1400, 1277, 1097, 1025, 732; MS: m/z (rel. intensity, %): 166 (M^+, 5), 149 (4), 139 (9), 124 (31), 108 (17), 94 (7), 80 (10), 71 (9), 57 (13), 45 (100), 41 (12); HRMS (FAB): $C_8H_{10}N_2O_2$. Found: 166.0732, calcd: 166.0742.

4.4.8. (*R*)-(+)-2-Hydroxy-2-(2-(*N*-methyl)pyrrolyl)acetonitrile 3d. Clear oil; yield: 17%; $[\alpha]_D^{21}$ +35.7 ($c=1.2$, $CHCl_3$); e.e. 40.1%; ^{1}H NMR ($CDCl_3$): δ, 2.90 (br, s, 1H, OH), 5.50 (s, 1H, Pyrr-H), 6.38 (m, 1H, Pyrr-H), 6.67 (m, 1H, Pyrr-H); IR (cm^{-1}): 3414 (w), 3114, 2953, 2248 (CN), 1644, 1543, 1494, 1417, 1300, 1024, 729; MS: m/z (rel. intensity, %): 137 (M^++1, 5), 136 (M^+, 56), 121 (M^+–CH_3, 2), 120 (M^+–1–CH_3, 9), 119 (M^++1–H_2O, 100), 109 (M^+–HCN, 49), 108 (M^+–1–HCN, 44), 91 (14), 80 (24), 65 (16), 57 (24), 53 (32), 41 (23); HRMS (FAB): $C_7H_8N_2O$. Found: 136.0613, calcd: 136.0637.

4.4.9. (*R*)-(+)-2-Hydroxy-2-(2-(5-acetyl-*N*-methyl)pyrrolyl)acetonitrile 3e. White solid, mp = 96–97°C; yield: 99%; $[\alpha]_D^{21}$ +38.8 ($c=0.80$, EtOH); e.e. 34.1%; ^{1}H NMR (acetone-d_6): δ, 2.40 (s, 3H, CH_3CO), 3.30 (s, 1H, OH), 3.96 (s, 3H, N-CH_3), 5.93 (s, 1H, CH), 6.37 (d, 1H, $J=4$ Hz, Pyrr-H), 7.03 (d, 1H, $J=4$ Hz, Pyrr-H); IR (cm^{-1}): 3337, 3200 (w), 3127, 2966, 2918, 2846, 2246, 1645, 1625, 1531, 1492, 1458, 1385, 1250, 1027, 768; MS: m/z (rel. intensity, %): 179 (M^++1, 5), 178 (M^+, 16), 163 (M^+–CH_3, 15), 151 (M^+–HCN, 100), 136 (M^+–CH_3–HCN, 78), 123 (14), 108 (19), 80 (22), 53 (23), 43 (19); elemental analysis: $C_9H_{10}N_2O_2$. Found: C, 60.5; H, 5.57; N, 15.78, calcd: C, 60.55; H, 5.66; N, 15.75%.

4.4.10. (*R*)-(+)-2-Hydroxy-2-(3-(5-cyano-*N*-methyl)pyrrolyl)acetonitrile 3f. White solid, mp = 104–105°C; yield: 84%; $[\alpha]_D^{21}$ +24.7 ($c=0.75$, EtOH); e.e. 66.4%; ^{1}H NMR (acetone-d_6): δ, 3.30 (s, 1H, OH), 3.82 (s, 3H, N-CH_3), 5.62 (s, 1H, CH), 6.96 (d, 1H, $J=1.8$ Hz, Pyrr-H), 7.26 (d, 1H, $J=1.8$ Hz, Pyrr-H); IR (cm^{-1}): 3406, 3125, 3093, 2954, 2881, 2225, 1561, 1484, 1414, 1398, 1214, 1135, 1052, 845, 600; MS: m/z (rel. intensity, %): 162 (M^++1, 8), 161 (M^+, 34), 144 (M^+–OH, 32), 135 (M^+–CN, 52), 134 (M^+–HCN, 65), 134 (M^+–H–HCN, 100), 117 (3), 105 (14), 78 (9), 64 (9); HRMS (FAB): $C_8H_7N_3O$. Found: 161.0896, calcd: 161.0859.

4.4.11. (*R*)-(+)-2-Hydroxy-2-(6-bromo-2-pyridinyl)acetonitrile 4c. Clear oil; yield: 92%; $[\alpha]_D^{21}$ +22.4 ($c=1.60$, $CHCl_3$); e.e. 65%; ^1H NMR ($CDCl_3$): δ, 4.4 (s, 1H, OH), 5.5 (s, 1H, CH), 7.3–7.4 (m, 3H, Ar-H); IR (cm^{-1}): 3105, 2927, 2682, 1589, 1562, 1456, 1435, 1306, 1262, 1245, 1163, 1137, 1075, 1001, 931, 792, 745, 649, 596; MS: m/z (rel. intensity, %): 212 (M^+, 19), 159 (98), 158 (48), 157 (100), 156 (34), 78 (42), 77 (20), 76 (29), 51 (24); HRMS (FAB): $C_7H_5BrN_2O$ (M^+–H): found: 211.9561, calcd: 211.9585.

4.4.12. (*R*)-(+)-2-Hydroxy-2-(3-pyridinyl)acetonitrile 4d. White crystal, mp = 85–86°C; yield 99.5%; $[\alpha]_D^{21}$ +21.4 ($c=0.95$, EtOH); e.e. 50%; ^1H NMR ($CDCl_3$): δ, 5.55 (br, s, 1H, OH), 5.65 (s, 1H, CH); 7.45 (dd, 1H, $J_1=8$ Hz, $J_2=1.6$ Hz, Py-H), 7.98 (dd, 1H, $J_1=8$ Hz, $J_2=1.6$ Hz, Py-H), 8.56 (d, 1H, $J=4.8$ Hz, Py-H), 8.61 (s, 1H, Py-H).

4.4.13. (*S*)-(−)-2-Hydroxy-2-(2-*N*-methylimidazolyl)-acetonitrile 5a. Colorless crystal, mp = 124°C (decomp.); yield: 94%; $[\alpha]_D^{21}$ −2 ($c=0.30$, MeOH); e.e. 5%; ^1H NMR (CD_3OD): δ, 4.0 (s, 4H, CH_3, OH), 6.03 (s, 1H, CH), 7.13 (d, 1H, $J=1.0$ Hz, Im-H), 7.35 (d, 1H, $J=1.0$ Hz, Im-H); IR (cm^{-1}): 2500–3300 (w), 3148, 3062, 2817, 2693, 2065, 1681, 1666, 1504, 1468, 1427, 1392, 1280, 1035, 925, 748, 719; MS: m/z (rel. intensity, %): 138 (M^++1, 11), 137 (M^+, 33), 120 (M^+–OH, 35), 111 (M^+–CN, 36), 110 (M^+–HCN, 83), 97 (11), 82 (100), 81 (72), 54 (61), 42 (66); elemental analysis: $C_6H_7N_3O$. Found: C, 52.23; H, 5.12; N, 30.72, calcd: C, 52.55; H, 5.15; N, 30.64%.

4.4.14. (*S*)-(+)-2-Hydroxy-2-(2-thiazolyl)acetonitrile 5b. Light yellow crystal, mp = 133–134°C; yield 97%; $[\alpha]_D^{21}$ +13 ($c=0.4$, EtOH); e.e. 67%; ^1H NMR (CD_3OD): δ, 6.17 (s, 1H, CH), 7.89 (AB, 1H, Ar-H, $J=3.3$ Hz), 8.03 (AB, 1H, Ar-H, $J=3.3$ Hz); IR (cm^{-1}): 3125 (w), 2843, 2725, 1508, 1454, 1408, 1187, 1123, 1046, 909, 753; MS: m/z (rel. intensity, %): 142 (M^++2, 4), 141 (M^++1, 15), 140 (M^+, 30), 123 (M^+–OH, 13), 114 (M^+–CN, 20), 110 (M^+–HCN, 26), 95 (8), 86 (68), 85 (51), 58 (100), 45 (21); elemental analysis: $C_5H_4N_2OS$. Found: C, 42.87; H, 2.78; N, 20.03, calcd: C, 42.85; H, 2.88; N, 19.98%.

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References

1. For examples, see: (a) Asselin, A. A.; Humber, L. G.; Crosilla, D.; Oshiro, G.; Wojdan, A.; Grimes, D.; Heaslip, R. J.; Rimele, T. J.; Shaw, C. C. *J. Med. Chem.* **1986**, *29*, 1009; (b) Naylor, E. M.; Colandrea, V. J.; Candelore, M. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3087.
2. For more recent reviews about the preparation of cyanohydrins catalyzed by oxynitrilases, see: (a) Effenberger, F.; Föster, S.; Wajant, H. *Curr. Opin. Biotechnol.* **2001**, 532 and references cited therein; (b) Gröger, H. *Adv. Synth. Catal.* **2001**, *343*, 547; (c) Gregory, R. J. H. *Chem. Rev.* **99**, *1999*, 3649 and references cited therein.
3. Brussee, J.; Loos, W. T.; Kruse, C. G.; van der Gen, A. *Tetrahedron* **1990**, *46*, 979.
4. (a) Han, S.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **1998**, *9*, 1835; (b) Han, S.; Lin, G.; Li, Z. *Tetrahedron* **1999**, *55*, 3531; (c) Han, S.; Chen, P.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 843.
5. Effenberger, F.; Eichhorn, J. *Tetrahedron: Asymmetry* **1997**, *8*, 469.
6. (a) Effenberger, F.; Ziegler, T.; Forster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458; (b) Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1555; (c) Effenberger, F. *Enantiomer* **1996**, *1*, 359; (d) Loos, W. T.; Geluk, H. W.; Ruijken, M. M. A.; Kruse, C. G.; Brussee, J.; van der Gen, A. *Biocatalysis and Biotransformation* **1995**, *12*, 255.
7. (a) Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. *Synth. Commun.* **1991**, *21*, 1387; (b) Huuhtanen, T. T.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1992**, *3*, 1223; (c) Kiljunen, E.; Karneva, L. T. *Tetrahedron: Asymmetry* **1996**, *7*, 1105; (d) Kiljunen, E.; Karneva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 1225; (e) Kiljunen, E.; Karneva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 1551.
8. (a) Schmidt, M.; Griengl, H. In *Oxynitrilases*: *From Cyanogenesis to Asymmetric Synthesis*; Fessner, W.-D., Ed. Topics in Current Chemistry; Springer-Verlag: Berlin, 1999; Vol. 200, pp. 193–226; (b) Schmidt, M.; Herve, S.; Klempier, N.; Griengl, H. *Tetrahedron* **1996**, *52*, 7833.
9. Ziegler, T.; Hörsch, B.; Effenberger, F. *Synthesis* **1990**, *7*, 575.