Pergamon

Tetrahedron, Vol. 52, No. 23, pp. 7833-7840, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00354-7

Preparation of Optically Active Cyanohydrins Using the (*S*)-Hydroxynitrile Lyase from *Hevea brasiliensis*†

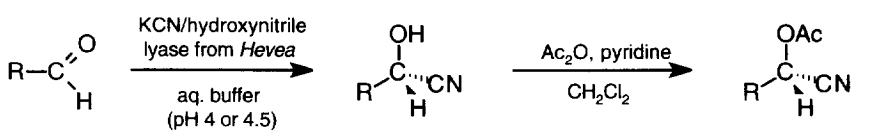
Michael Schmidt, Stephanie Hervé, Norbert Klempier and Herfried Griengl*

Institut für Organische Chemie der Technischen Universität Graz, Spezialforschungsbereich Biokatalyse, Stremayrgasse 16, A-8010 Graz, Austria

Abstract: Several aliphatic, aromatic and heteroaromatic aldehydes have been converted into the chiral cyanohydrins using the (*S*)-hydroxynitrile lyase from *Hevea brasiliensis*. The corresponding cyanohydrins were obtained in moderate to good yield and high enantiomeric excess with the exeption of phenyloxyacetaldehyde, benzyloxyacetaldehyde and the pyrrole-, pyridine- and indolealdehydes investigated. In contrast to previously reported results, cinnamaldehyde could be converted into (*S*)-(-)-2-hydroxy-4-phenyl-(*E*)-but-3-enenitrile with good selectivity by means of optimized reaction conditions. Copyright © 1996 Elsevier Science Ltd

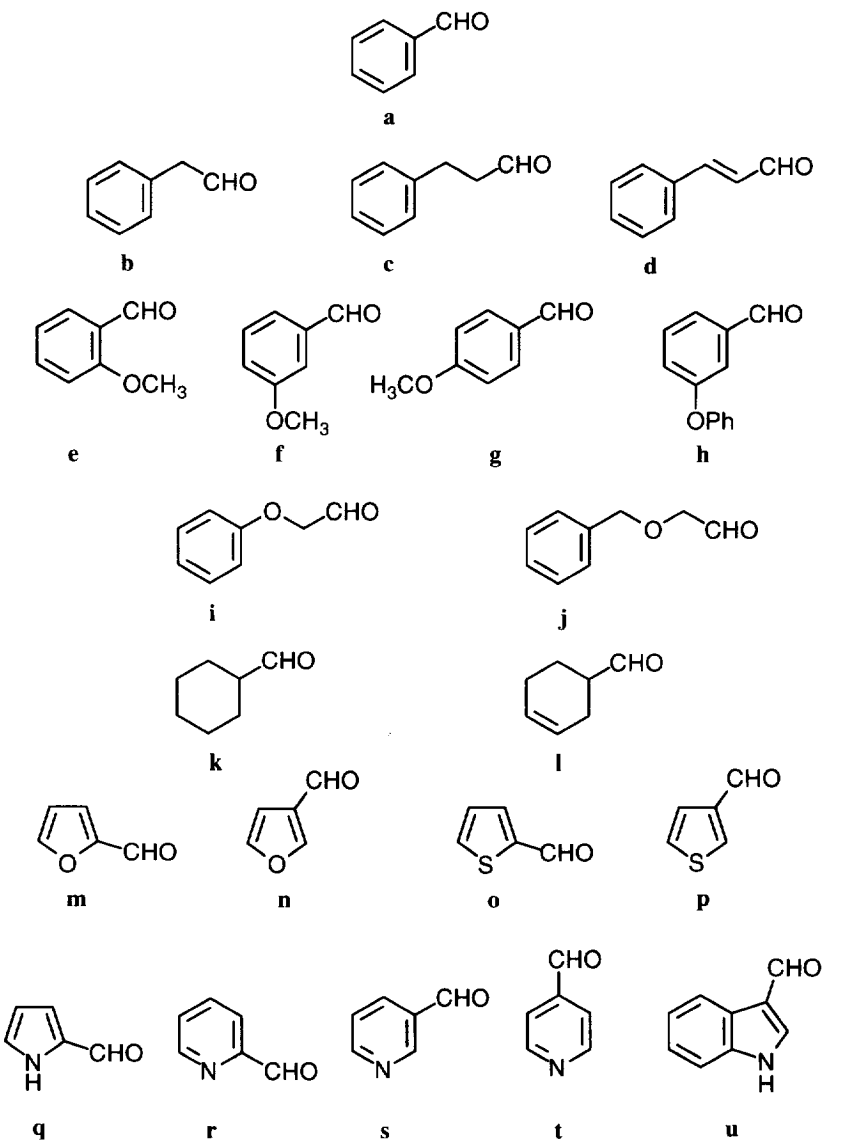
Cyanohydrins are of broad interest as starting materials for the preparation of several important classes of compounds such as α-hydroxyacids and -esters, α-aminoacids, β-aminoalcohols, α-hydroxyaldehydes, vicinal diols and α-hydroxyketones.[1-4] Due to the increased demand for the availability of chiral compounds as pure enantiomers a number of methods for the preparation of enantiopure cyanohydrins have been developed using various chiral catalysts, such as cyclic dipeptides,[5-7] chiral complexes with titanium,[8,9] aluminium[10] and boron[11] or enzymes for resolution.[12-16] Also the enantioselective synthesis of cyanohydrins has been performed enzymatically by means of hydroxynitrile lyases (oxynitrilases) from different plant sources.[17-21]

We have recently shown that the hydroxynitrile lyase from *Hevea brasiliensis* is an efficient catalyst for the preparation of aliphatic (*S*)-cyanohydrins.[22,23] To explore scope and limitations of this reaction a broad range of aldehydes has been investigated (see Scheme 1) using as enzyme a crude recombinant protein, which was prepared by overexpression in *Pichia pastoris*.[24] To avoid the handling of free hydrogen cyanide, KCN and a citric acid buffer (pH 4.0 or 4.5), which generates HCN *in situ*, was applied. Under these conditions the spontanous addition of HCN to the aldehyde in most cases is almost completely suppressed, which leads to a high



Scheme 1.

optical purity of the corresponding cyanohydrin. In order to obtain stable compounds for structural analysis and for follow up reactions of our interest, the cyanohydrins were acetylated. Unless otherwise indicated, optimized reaction conditions were employed (see Experimental Part). The data shown in Table 1 suggest, that the synthesis of aromatic and heteroaromatic cyanohydrins can be accomplished with excellent enantiomeric purity. In particular, the superior selectivity for furan-2- (**1m**), thiophene-2- (**1o**) and -3-aldehyde (**1p**) in comparison to results obtained by using (*S*)-hydroxynitrile lyases from other plant sources[19,21,25] is striking.



Scheme 2. Aldehydes investigated

The aldehydes **1f** and **1g**, which are substituted either in the 3- or 4-position of the aromatic ring, gave the corresponding chiral cyanohydrins with no significant differences in optical purity. In contrary, 2-methoxybenzaldehyde (**1e**) could be converted into its cyanohydrin only with relatively low enantiomeric excess, which might be explained by an unfavorable steric interaction of this substrate with the catalytic site of the enzyme. 3-Phenoxybenzaldehyde (**1h**) is transformed by the enzyme from *Hevea brasiliensis* with good enantiomeric excess, although it is an aldehyde which reacts slowly, presumably because of its low solubility in the aqueous system.

Table 1. Enantioselective formation of cyanohydrins using (*S*)-hydroxynitrile lyase from *Hevea brasiliensis*.

aldehyde	yield(%)	e.e.(%)[a]	aldehyde	yield(%)	e.e.(%)	aldehyde	yield(%)	e.e.(%)
a	67	>99[b]	**h**	9	99	**o**	52	99[b,d]
b	44	99	**i**	n.d.[e]	0	**p**	49	99[b,d]
c	88	93	**j**	n.d.	0	**q**	0	0
d	50	95	**k**	94	99	**r**	n.d.	0
e	61	77	**l**	87	99[c]	**s**	n.d.	0
f	80	99	**m**	55	98[b,d]	**t**	n.d.	0
g	49	95	**n**	61	99[b,f]	**u**	0	0

[*a*] all (*S*)-configurated. [*b*] Standard conditions were employed (see Experimental Part). [*c*] diastereomeric excess, because aldehyde **1l** was racemic. [*d*] (*R*)-configuration has to be assigned according to Cahn-Ingold-Prelog (CIP). [*e*] not determined. [*f*] configuration not determined.

The cyanohydrins of pyridine-2- (**1r**), -3- (**1s**) and -4-aldehyde (**1t**) were found to be racemic although their corresponding aldehydes are perfectly soluble in the aqueous reaction medium used. However, blank experiments without the enzyme present in the reaction mixture, also resulted in formation of the (racemic) cyanohydrins **2r**, **2s** and **2t.** This indicates that the carbonyl group of the tested pyridine aldehydes is highly activated, which leads to an increased rate of the spontanous addition of HCN and therefore to a complete loss of optical purity. However, Effenberger *et al.*[3] were able to increase the enantiomeric excess of the cyanohydrin derived from nicotinic aldehyde up to 82% using an immobilized (*R*)-hydroxynitrile lyase from *Prunus amygdalis* in organic solvents.

Compound **1u** is only partially soluble in the citric acid buffer solution and this may be the reason that no conversion was observed. Hörsch[25] was unable to transform this aldehyde as well, although an immobilized (*R*)-hydroxynitrile lyase in organic solvents has been used in this case. Pyrrol-2-aldehyde (**1q**) was also not converted, although it is nearly perfectly soluble in the aqueous citric acid buffer. It is known,[3,7,25,27] that some nitrogen containing heteroaromatic carbonyl compounds are difficult to convert into the corresponding optically enriched cyanohydrins. This observation is so far not clearly understood and therefore has to be further investigated.

Phenoxy- (**1i**) and benzyloxyacetaldehyde (**1j**) showed moderate solubility in citric acid buffer, the corresponding cyanohydrins were found to be racemic. Regarding **1i** the same observation has already been made using a (*R*)-hydroxynitrile lyase preparation from *Prunus amygdalis* in ethanol/acetate buffer.[18b] Other

arylsubstituted aliphatic aldehydes are transformed into the corresponding cyanohydrins by using the (*S*)-hydroxynitrile lyase from *Hevea brasiliensis* with good enantioselectivity. Even 3-phenylpropanal (**1c**), which is difficult to convert with good yield and optical purity using standard reaction conditions (see Experimental Part), could be reacted to give the corresponding cyanohydrin with at least 93% e.e. and 88% yield after optimizing the reaction conditions in aqueous buffer solution. In contrast to previous observations,[18b,23] cinnamaldehyde (**1d**) could be converted into the corresponding (*S*)-cyanohydrin with good selectivity by means of optimized reaction conditions. However, in contrary to all the other substrates tested, cinnamaldehyde cyanohydrin (**2d**) showed a significant rate of racemization during the derivatization into its acetate. Therefore we prepared the TBDMS derivate (**3d'**) of this compound, which proceeded with no noteworthy loss of optical purity. Recently, also a method for the preparation of the (*R*)-cyanohydrin derived from cinnamaldehyde catalyzed by a (*R*)-hydroxynitrile lyase has been developed.[33]

The alicyclic aldehydes **1k** and **1l** are known to convert into the corresponding cyanohydrins with good optical purities by means of the (*R*)-hydroxynitrile lyase from *Prunus amygdalis*.[25,29] In contrast to previous observations with the hydroxynitrile lyase from *Sorghum bicolor*,[25] which shows (*S*)-selectivity, we were able to prepare the (*S*)-cyanohydrins of both **1k** and **1l** with good yield and enantiomeric excess.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz or on a Bruker MSL 300 MHz instrument in $CDCl_3$ using tetramethylsilane as an internal standard. IR spectra were recorded on a Bomem Michelson 100 instrument, MS spectra on a Kratos Profile sector field instrument (70 eV). Optical rotations were measured using a Jasco DIP 370 polarimeter. Determination of the enantiomeric excess was accomplished by gas chromatographic resolution of the corresponding acetates on a 25m x 0.32 mm Chirasil-DEX-CB fused silica capillary column (0.25 µm; carrier gas hydrogen). In case of compound **2d**, the corresponding TBDMS ether **3d'** was used for measuring the e.e., employing a CHIRACEL OD-H HPLC-column (25 x 0.46 cm) with n-heptane/2-propanol 99/1 (v/v) as the eluent with a flow rate of 0.6 ml/min and UV detection (254 nm).

Chemicals

In all cases commercially available products were used. Aldehydes with a purity of less than 99% were distilled in an inert atmosphere prior to use.

Enzyme preparation

A recombinant (*S*)-hydroxynitrile lyase, which is homologous to the natural enzyme from *Hevea brasiliensis*, was prepared by overexpression in *Pichia pastoris* . A crude cytoslic extract was used for all biotransformations.[24]

Enzyme catalyzed preparation of cyanohydrins and the corresponding acetates

1. Standard conditions

To a solution of 3 mmol aldehyde in 5 ml 0.1 M sodium citrate buffer (pH 4.0) were added 300 IU of crude enzyme extract (140 IU/ml) and the mixture was cooled down to ice bath temperature. Subsequently 2 mole equivalents of potassium cyanide, adjusted to pH 4.0 with cold 0.1 M citric acid (45 ml), were added dropwise within 20 min under continuous cooling. Stirring at 0 - 5°C was continued for additional 40 min, followed by extraction of the product with methylene chloride (3 x 50 ml). After drying over anhydrous sodium sulfate and evaporation of the solvent, the crude cyanohydrin was obtained, which was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (5/1 or 9/1) acidified with a trace amount of anhydrous HCl as the eluent. Unless otherwise mentioned, all cyanohydrins isolated were colorless to slightly yellow oils. Crystallization of the free cyanohydrins, which may alter the enantiomerical composition, was not attempted.

After measuring the optical rotation of the free cyanohydrin, the compound was converted directly into the acetate by reaction with 2 mole equivalents of acetic anhydride and pyridine in methylene chloride (20 ml) at room temperature overnight. Washing the organic layer with 5% H_2SO_4, distilled water and saturated $NaHCO_3$ (20 ml each), drying with anhydrous sodium sulfate and concentrating the reaction mixture *in vacuo* followed by flash chromatography in petroleum ether/ethyl acetate (5/1 or 9/1) yielded the pure acetylated cyanohydrins as colorless to slightly yellow oils, which were used directly for structural analysis.

(S)-(-)-2-Hydroxy-2-phenylacetonitrile (2a). $[\alpha]_D^{20}$ -46.5° (c 1.4, $CHCl_3$); Lit.[18b]: $[\alpha]_D^{20}$ +45° (c 1, $CHCl_3$) for the (*R*)-isomer with e.e.>99%. Isolated yield (after chromatography): 67%.

(S)-(-)-2-Acetoxy-2-phenylacetonitrile (3a). $[\alpha]_D^{20}$ -7.24° (c 2.3, $CHCl_3$); Lit.[30]: $[\alpha]_D^{20}$ +7.8° (c 5, $CHCl_3$) for the (*R*)-isomer with 97% e.e.. Isolated yield (after chromatography): 89%; e.e.>99%. -^{1}H NMR: δ(ppm) 2.16 (s, 3H, CH_3CO); 6.42 (s, 1H, CH); 7.48 (m, 5H, aromatic). -^{13}C NMR: 168.87 (C=O); 131.80 (C-1); 130.48 (C-4); 129.32 (C-3,5); 127.94 (C-2,6); 116.12 (CN); 62.92 (CH); 20.54 (CH_3CO). -IR: 3040, 2950, 1750, 1380, 1220, 1020, 700 cm^{-1}. -MS: m/z (rel. intensity %): 175 (M^+,42), 133 (100), 116 (65), 115 (75), 105 (33), 89 (20), 77 (15), 63 (10), 51 (10), 43 (55), 39 (6).

(R)-(-)-2-(2-Furyl)-2-hydroxyacetonitrile (2m). $[\alpha]_D^{20}$ -27.3° (c 2.3, $CHCl_3$); Lit.[7]: $[\alpha]_D^{25}$ +14° (c 1.8698, $CHCl_3$) for the (*S*)-isomer with 79% e.e.. Isolated yield (after chromatography): 55%.

(R)-(+)-2-Acetoxy-2-(2-furyl)acetonitrile (3m). $[\alpha]_D^{20}$ +24.3° (c 1.6, $CHCl_3$); Lit.[26]: $[\alpha]_D^{25}$ +12.8° (c 1.02, $CHCl_3$) for the (*R*)-isomer with 47% e.e.. Isolated yield (after chromatography): 86%; e.e.=98%. -^{1}H NMR: δ (ppm) 2.17 (s, 3H, CH_3CO); 6.45 (dd, 1H, J=1.83 Hz, J=3.34 Hz, furyl); 6.47 (s, 1H, CH); 6.68 (dd, 1H, J=3.34 Hz, J=0.50 Hz, furyl); 7.51 (dt, 1H, J=1.85 Hz, J=0.92 Hz, furyl). -^{13}C NMR: 168.68 (C=O); 145.10, 144.03, 112.64, 111.18 (furyl); 114.17 (CN); 55.77 (CH); 20.38 (CH_3CO). -IR: 3140, 2950, 1760, 1370, 1210, 1010, 750 cm^{-1}. -MS: m/z (rel. intensity %): 165 (M^+, 38), 123 (55), 106 (100), 105 (52), 95 (12), 77 (62), 68 (5), 51 (24), 43 (95), 39 (13), 32 (3), 28 (30).

(-)-2-(3-Furyl)-2-hydroxyacetonitrile (2n). $[\alpha]_D^{20}$ -14.8° (c 2.25, $CHCl_3$). Isolated yield (after chromatography): 61%.

(-)-2-Acetoxy-2-(3-furyl)acetonitrile (3n). $[\alpha]_D^{20}$ -11.6° (c 2.0, $CHCl_3$). Isolated yield (after chromatography): 80%; e.e.=98.5%. -^{1}H NMR: δ(ppm) 2.16 (s, 3H, CH_3CO); 6.36 (s, 1H, CH); 6.54 (dd, 1H, J=1.87 Hz, J=0.83 Hz, furyl); 7.47 (dd, 1H, J=1.79 Hz, J=1.69 Hz, furyl); 7.68 (m, 1H, furyl). -^{13}C NMR: 169.15 (C=O); 144.77, 142.75 (C-2', C-5'); 118.34, 109.46 (C-3', C-4'); 115.86 (CN); 55.78 (CH); 20.63 (CH_3CO). -IR: 3140, 2950, 1760,1370, 1220, 1160, 1020, 870, 600 cm^{-1}. -MS: m/z (rel. intensity %): 165 (M^+, 3), 123 (25), 106 (29), 95 (8), 77 (6), 51 (12), 43 (100), 39 (5), 32 (4), 28 (25).

(R)-(-)-2-Hydroxy-2-(2-thienyl)acetonitrile (2o). $[\alpha]_D^{20}$ -61.3° (c 2.0, $CHCl_3$); Lit.[7]: $[\alpha]_D^{25}$ +46.8° (c 2.515, $CHCl_3$) for the (*S*)-isomer with 58% e.e.. Isolated yield (after chromatography): 52%.

(R)-(+)-2-Acetoxy-2-(2-thienyl)acetonitrile (3o). $[\alpha]_D^{20}$ +10.4° (c 0.3, $CHCl_3$); Lit.[30]: $[\alpha]_D^{20}$ -8.3° (c 5, $CHCl_3$) for the (*S*)-isomer with 85% e.e.. Isolated yield (after chromatography): 83%; e.e.=99%. -^{1}H NMR: δ(ppm) 2.17 (s, 3H, CH_3CO); 6.63 (s, 1H, CH); 7.05 (dd, 1H, J=3.64 Hz, J=5.13 Hz, thienyl); 7.35 (ddd, 1H, J=1.27 Hz, J=3.64 Hz, J=0.67 Hz, thienyl); 7.42 (dd, 1H, J=1.28 Hz, J=5.12 Hz, thienyl). -^{13}C NMR: 168.97 (C=O); 133.80, 129.79, 129.20, 127.47 (thienyl); 115.66 (CN); 58.34 (CH); 20.63 (CH_3CO). -IR: 3100, 2940, 1760, 1370, 1210, 1020, 720 cm^{-1}. -MS: m/z (rel. intensity %): 181 (M^+, 50), 139 (32), 122 (87), 121 (100), 111 (18), 95 (6), 84 (6), 78 (6), 69 (7), 58 (10), 51 (7), 45 (30), 43 (85), 39 (18), 32 (3), 28 (12).

(R)-(-)-2-Hydroxy-2-(3-thienyl)acetonitrile (2p). $[\alpha]_D^{20}$ -42.6° (c 1.65, $CHCl_3$); Lit.[25]: $[\alpha]_D^{20}$ -45.1° (c 0.71, $CHCl_3$) for the (*R*)-isomer with 87% e.e.. Isolated yield (after chromatography): 49%.

(R)-(+)-2-Acetoxy-2-(3-thienyl)acetonitrile (3p). $[\alpha]_D^{20}$ +12.2° (c 1.45, $CHCl_3$). Isolated yield (after chromatography): 77%; e.e.=99%. -^{1}H NMR: δ(ppm) 2.16 (s, 3H, CH_3CO); 6.48 (s, 1H, CH); 7.20 (dd, 1H, J=5.09 Hz, J=1.28 Hz, thienyl); 7.42 (dd, 1H, J=5.13 Hz, J=2.97 Hz, thienyl); 7.59 (ddd, 1H, J=2.99 Hz, J=1.30 Hz, J=0.67 Hz, thienyl). -^{13}C NMR: 169.05 (C=O); 132.43, 127.96, 126.70, 126.47 (thienyl); 116.12 (CN); 58.46 (CH); 20.56 (CH_3CO). -IR: 3110, 1750, 1220, 1020, 790 cm^{-1}. -MS: m/z (rel. intensity %): 181 (M^+, 15), 139 (95), 122 (57), 121 (70), 111 (15), 95 (6), 84 (4), 78 (4), 69 (4), 58 (6), 51 (6), 45 (28), 43 (100), 39 (15), 32 (5), 28 (21).

2. Optimized experiments

For substrates resulting in an enantiomeric excess less than 95% using standard conditions, optimized reaction conditions were employed.

To a stirred solution of 1 mmol aldehyde in 1.7 ml of 0.1 M sodium citrate buffer (pH 4.5) were added 2000 IU of (*S*)-hydroxynitrile lyase, and the mixture was cooled down to ice bath temperature. Subsequently 2.5 mole equivalents of potassium cyanide adjusted to pH4.5 with cold 0.1M citric acid (17 ml), were added in one portion. After stirring for 1h at 0-5°C, the reaction mixture was extracted with methylene chloride (3 x 50 ml). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was removed by evaporation. The crude cyanohydrin was purified by column chromatography using petroleum ether/ethyl acetate (5/1 or 9/1) acidified with trace amounts of anhydrous HCl as the eluent. Unless otherwise mentioned, all cyanohydrins isolated were colorless to slightly yellow oils. Crystallization of the free cyanohydrins, which may alter the enantiomerical composition, was not attempted. After measuring the optical rotation of the pure

cyanohydrins, the compounds were converted directly into the corresponding acetates by reaction with 2 mole equivalents of acetic acid anhydride and pyridine in methylene chloride (20 ml) at room temperature overnight. Washing the organic layer with 5% H_2SO_4, distilled water and saturated $NaHCO_3$ (each 20 ml), drying with anhydrous sodium sulfate and concentrating the reaction mixture *in vacuo* followed by column chromatography in petroleum ether/ ethyl acetate (5/1 or 9/1) yielded the pure acetylated cyanohydrins as colorless to slightly yellow oils, which were used for further analysis.

*(S)-(-)-2-Hydroxy-3-phenylpropanenitrile (**2b**).* $[\alpha]_D^{20}$ -7.73° (c 2.75, $CHCl_3$); Lit.[29]: $[\alpha]_D^{20}$ +6.8° (c 0.38, $CHCl_3$) for the (*R*)-isomer with 88% e.e.. Isolated yield (after chromatography): 44%.

*(S)-(-)-2-Acetoxy-3-phenylpropanenitrile (**3b**).* $[\alpha]_D^{20}$ -61.5° (c 1.5, $CHCl_3$); Lit.[32]: $[\alpha]_D^{20}$ -52.2° (c 1.16, $CHCl_3$) for the (*S*)-isomer derived from the corresponding α-acetoxycarboxylic acid with 91% e.e.. Isolated yield (after chromatography): 90%; e.e.=99%. -^{1}H NMR: δ(ppm) 2.12 (s, 3H, CH_3CO); 3.20 (d, 2H, CH_2); 5.50 (t, 1H, CH); 7.28-7.41 (m, 5H, aromatic). -^{13}C NMR: 169.08 (C=O); 133.53, 129.71, 129.03, 128.09 (aromatic); 166.64 (CN); 62.03 (CH); 38.77 (CH_2); 20.44 (CH_3CO). -IR: 3030, 2940, 1760, 1220, 1040, 700 cm^{-1}. -MS: m/z (rel. intensity %): 189 (M^+, 0.3), 130 (15), 129 (97), 103 (5), 91 (100), 77 (8), 65 (12), 51 (9), 43 (67), 39 (8), 32 (5), 28 (27).

*(S)-(+)-2-Hydroxy-4-phenylbutanenitrile (**2c**).* $[\alpha]_D^{20}$ +7.05° (c 1.3, $CHCl_3$); Lit.[8]: $[\alpha]_D^{20}$ -6.79° (c 2.04, $CHCl_3$) for the (*R*)-isomer with 89% e.e.. Isolated yield (after chromatography): 88%.

*(S)-(-)-2-Acetoxy-4-phenylbutanenitrile (**3c**).* $[\alpha]_D^{20}$ -43.4° (c 1.95, $CHCl_3$); Lit.[12]: $[\alpha]_D^{20}$ -43.8° (1≤c≤2, $CHCl_3$) for the (*S*)-isomer with 90% e.e.. Isolated yield (after chromatography): 86%; e.e.=93%. -^{1}H NMR: δ(ppm) 2.13 (s, 3H, CH_3CO); 2.25 (q, 2H, CH_2); 2.85 (t, 2H, CH_2); 5.28 (t, 1H, CH); 7.21- 7.37 (m, 5H, aromatic). -^{13}C NMR: 169.13 (C=O); 139.31, 128.90, 128.48, 126.80 (aromatic); 116.89 (CN); 60.72 (CH); 33.89 (CH_2); 30.88 (CH_2); 20.34 (CH_3CO). -IR: 3030, 2940, 1760, 1220, 1040, 700 cm^{-1}. -MS: m/z (rel. intensity %): 203 (M^+, 5), 143 (100), 116 (13), 105 (8), 91 (20), 77 (4), 65 (4), 51 (3), 43 (17).

*(S)-(-)-2-Hydroxy-4-phenyl-(E)-but-3-enenitrile (**2d**).* $[\alpha]_D^{20}$ -27.9° (c 1.95, $CHCl_3$); Lit.[28]: $[\alpha]_D^{26}$ -19.0° (c 1, $CHCl_3$) for the (*S*)-isomer with 62% e.e.. Isolated yield (after chromatography): 50% (slightly yellow solid).

*(S)-(-)-2-[(tert-Butyldimethylsilyl)oxy]-4-phenyl-(E)-but-3-enenitrile (**3d'**).* $[\alpha]_D^{20}$ -6.27° (c 0.35, $CHCl_3$). Isolated yield (after chromatography): 95%; e.e.=95%. -^{1}H NMR: δ(ppm) 0.21 (s, 3H, CH_3); 0.24 (s, 3H, CH_3); 0.97 (s, 9H, $C(CH_3)_3$); 5.14 (d, 1H, J=5.78 Hz, CH-CN); 6.20 (dd, 1H, J=15.3 Hz, J=5.79 Hz, C=CH-C-CN); 6.83 (d, 1H, J=15.7 Hz, Ph-CH=C); 7.33-7.44 (m, 5H, aromatic). -^{13}C NMR: 135.17 (aromatic); 133.71 (C=C); 128.83, 127.04 (aromatic); 123.77 (C=C); 118.55 (CN); 62.73 (CH); 25.63 ($C(CH_3)_3$); 18.26 (C-Si); -4.87, -4.92 (CH_3-Si). -IR: 2940, 2860, 1470, 1260, 1100, 980, 840, 780, 690 cm^{-1}. -MS: m/z (rel. intensity %): 273 (M^+, 18), 258 (4), 217 (26), 216 (100), 190 (16), 189 (69), 166 (11), 142 (49), 131 (8), 115 (72), 103 (5), 84 (6), 75 (27), 57 (8), 41 (7).

*(S)-(-)-2-Hydroxy-2-(2-methoxyphenyl)acetonitrile (**2e**).* $[\alpha]_D^{20}$ -21.0° (c 1.25, $CHCl_3$); Lit.[7]: $[\alpha]_D^{25}$ +2.7° (c 2.354, $CHCl_3$) for the (*R*)-isomer with 56% e.e.. Isolated yield (after chromatography): 61%.

*(S)-(-)-2-Acetoxy-2-(2-methoxyphenyl)acetonitrile (**3e**).* $[\alpha]_D^{20}$ -19.6° (c 1.6, $CHCl_3$); Lit.[30]: $[\alpha]_D^{20}$ +19.7° (c 5, $CHCl_3$) for the (*R*)-isomer with 89% e.e.. Isolated yield (after chromatography): 96%; e.e.=77%. -^{1}H NMR: δ(ppm) 2.17 (s, 3H, CH_3CO); 3.88 (s, 3H, OCH_3); 6.71 (s, 1H, CH); 6.91-7.09, 7.39-7.59 (m, 4H, aromatic). -^{13}C NMR: 169.01 (C=O); 156.78, 131.87, 128.83, 120.99, 116.32, 111.14 (aromatic); 119.99 (CN); 58.22 (CH); 55.79 (OCH_3); 20.52 (CH_3CO). -IR: 2950, 1750, 1490, 1260, 1210, 1030, 960, 760 cm^{-1}. -MS: m/z (rel. intensity %): 205 (M^+, 25), 163 (100), 146 (25), 130 (30), 116 (32), 103 (11), 91 (17), 77 (18), 63 (7), 51 (11), 43 (75), 39 (10), 28 (19).

*(S)-(-)-2-Hydroxy-2-(3-methoxyphenyl)acetonitrile (**2f**).* $[\alpha]_D^{20}$ -40.8° (c 1.35, $CHCl_3$); Lit.[25]: $[\alpha]_D^{20}$ -38.1° (c 0.48, $CHCl_3$) for the (*S*)-isomer with 89% e.e.. Isolated yield (after chromatography): 80%.

*(S)-(-)-2-Acetoxy-2-(3-methoxyphenyl)acetonitrile (**3f**).* $[\alpha]_D^{20}$ -4.76° (c 2.05, $CHCl_3$); Lit.[30]: $[\alpha]_D^{20}$ +5.0° (c 5, $CHCl_3$) for the (*R*)-isomer with 98% e.e.. Isolated yield (after chromatography): 90%; e.e.=99%. -^{1}H NMR: δ(ppm) 2.17 (s, 3H, CH_3CO); 3.84 (s, 3H, Ph-OCH_3); 6.38 (s, 1H, CH); 6.95-7.11, 7.32-7.40 (m, 4H, aromatic). -^{13}C NMR: 168.97 (C=O); 160.16, 133.07, 130.42, 120.03, 116.09, 113.32 (aromatic); 120.12 (CN); 62.78 (CH); 55.49 (OCH_3); 20.54 (CH_3CO). -IR: 2950, 2840, 1760, 1600, 1490, 1220, 1030, 780, 690 cm^{-1}. -MS: m/z (rel. intensity %): 205 (M^+, 18), 163 (100), 146 (18), 136 (7), 116 (11), 103 (5), 89 (5), 77 (8), 63 (4), 51 (5), 43 (43), 39 (5), 28 (25).

*(S)-(-)-2-Hydroxy-2-(4-methoxyphenyl)acetonitrile (**2g**).* $[\alpha]_D^{20}$ -45.5° (c 1.5, $CHCl_3$); Lit.[18b]: $[\alpha]_D^{20}$ +49° (c 1, $CHCl_3$) for the (*R*)-isomer with 99% e.e.. Isolated yield (after chromatography): 49%.

*(S)-(+)-2-Acetoxy-2-(4-methoxyphenyl)acetonitrile (**3g**).* $[\alpha]_D^{20}$ +19.0° (c 1.55, $CHCl_3$); Lit.[30]: $[\alpha]_D^{20}$ -17.5° (c 5, $CHCl_3$) for the (*R*)-isomer with 90% e.e.. Isolated yield (after chromatography): 93%; e.e.=95%. -^{1}H NMR: δ(ppm) 2.14 (s, 3H, CH_3CO); 3.83 (s, 3H, OCH_3); 6.35 (s, 1H, CH); 6.93-6.97 (s, 2H, aromatic), 7.43-7.47 (s, 2H, aromatic). -^{13}C NMR: 169.22 (C=O); 161.46, 129.87, 124.20, 114.87 (aromatic); 116.57 (CN); 62.86 (CH); 55.67 (OCH_3); 20.72 (CH_3CO). -IR: 2950, 2840, 1750, 1610, 1510, 1250, 1220, 1180, 1030, 960, 830, 570 cm^{-1}. -MS: m/z (rel. intensity %): 205 (M^+, 35), 163 (32), 146 (92), 145 (100), 135 (15), 130 (5), 116 (10), 103 (12), 91 (8), 76 (12), 64 (6), 50 (8), 43 (35), 39 (7), 28 (18).

*(S)-(-)-2-Hydroxy-2-(3-phenoxyphenyl)acetonitrile (**2h**).* $[\alpha]_D^{20}$ -29.4° (c 0.4, $CHCl_3$); Lit.[28]:$[\alpha]_D^{20}$ -24.4° (c 2, $CHCl_3$) for the (*S*)-isomer with 91% e.e.. Isolated yield (after chromatography): 9%.

*(S)-(+)-2-Acetoxy-2-(3-phenoxyphenyl)acetonitrile (**3h**).* $[\alpha]_D^{20}$ +7.44° (c 0.75, $CHCl_3$); Lit.[26]: $[\alpha]_D^{27}$ +21.3° (c 10.27, benzene) for the (*S*)-isomer with 83% e.e.. Isolated yield (after chromatography): 92%; e.e.=99%. -^{1}H NMR: δ(ppm) 2.18 (s, 3H, CH_3CO); 6.36 (s, 1H, CH); 7.01- 7.44 (m, 9H, aromatic). -^{13}C NMR: 169.00 (C=O); 158.52 (Ph-O); 156.52 (Ph-O); 133.80, 130.88, 130.24, 124.35, 122.33, 120.36, 119.64, 117.96 (aromatic); 116.12 (CN); 62.69 (CH); 20.66 (CH_3CO). -IR: 3060, 1760, 1590, 1490, 1220, 1030, 690 cm^{-1}. -MS: m/z (rel. intensity %): 267 (M^+, 18), 225 (45), 198 (15), 181 (7), 141 (8), 114 (16), 87 (9), 77 (19), 71 (28), 51 (17), 43 (100), 28 (31).

*(S)-(-)-2-Cyclohexyl-2-hydroxyacetonitrile (**2k**).* $[\alpha]_D^{20}$ -9.83° (c 3.25, $CHCl_3$); Lit.[28]: $[\alpha]_D^{20}$ +4.7° (c 3.82, $CHCl_3$) for the (*R*)-isomer with 54% e.e.. Isolated yield (after chromatography): 94%.

*(S)-(-)-2-Acetoxy-2-cyclohexylacetonitrile (**3k**).* $[\alpha]_D^{20}$ -65.0° (c 1.05, CH_2Cl_2); Lit.[31]: $[\alpha]_D^{20}$ -57.7° (c 0.83, CH_2Cl_2) for the (*S*)-isomer with 96.8% e.e.. Isolated yield (after chromatography): 95 %; e.e.=99%. -^{1}H NMR: δ(ppm) 1.11-1.30 (m, 5H, cyclohexyl); 1.69-1.91 (m, 6H, cyclohexyl); 2.13 (s, 3H, CH_3CO); 5.16 (d, 1H, CH). -^{13}C NMR: 169.35 (C=O); 116.35 (CN); 65.77 (CH); 40.21, 28.30, 28.08, 25.94, 25.54, 25.47 (cyclohexyl); 20.49 (CH_3CO). -IR: 2930, 2860, 1760, 1450, 1370, 1220, 1030 cm^{-1}. -MS: m/z (rel. intensity %): 181 (M^+, 1.8), 139 (10), 99 (62), 83 (100), 67 (8), 57 (15), 55 (61), 43 (58), 41 (38), 28 (15).

*(S)-(-)-2-(Cyclohex-3-en-yl)-2-hydroxyacetonitrile (**2l**).* $[\alpha]_D^{20}$ -12.3° (c 1.40, $CHCl_3$); Lit.[25]: $[\alpha]_D^{20}$ +9.60° (c 1.2, $CHCl_3$) for the (*R*)-isomer with 93% e.e.. Isolated yield (after chromatography): 87%.

*(S)-(-)-2-Acetoxy-2-(cyclohex-3-enyl)acetonitrile (**3l**).* $[\alpha]_D^{20}$ -66.0° (c 2.65, $CHCl_3$); Lit.[31]: $[\alpha]_D^{20}$ -50.4° (c 0.645, CH_2Cl_2) for the (*S*)-isomer with 94.3% e.e.. Isolated yield (after chromatography): 93%; de>99%. -^{1}H NMR: δ(ppm) 1.42-1.60 (m, 1H, CH); 1.84-2.30 (m, 6H, cyclohexyl); 2.15 (s, 3H, CH_3CO); 5.26 (d, 1H, CH); 5.62-5.75 (m, 2H, olefinic). -^{13}C NMR: 169.37 (C=O); 127.32, 124.59 (olefinic); 116.28 (CN); 65.17 (CH); 36.63, 26.93, 24.44, 24.18 (cyclohexyl); 20.50 (s, 3H, CH_3CO). -IR: 3030, 2930, 2840, 1750, 1370, 1220, 1040 cm^{-1}. -MS: m/z (rel. intensity %): 179 (M^+, 0.7), 119 (57), 118 (54), 104 (20), 92 (30), 81 (51), 79 (58), 78 (17), 66 (9), 53 (15), 43 (100), 41 (16), 39 (18), 28 (20).

Acknowledgement: Stephanie Hervé gratefully acknowledges receiving a grant from the ERASMUS-exchange program. We wish to thank Gerhild Harter for carrying out the enzyme preparation and Prof. Walter Steiner for his useful advice in performing the reaction optimization. We also thank Carina Illaszewicz for recording the spectra. This work was financially supported by the Austrian Science Foundation and by the Christian Doppler Society.

REFERENCES AND NOTES

†Dedicated to Professor Miha Tišler on the occasion of his 70th birthday.

1. Kruse, C. G. Chiral Cyanohydrins-Their Manufacture and Utility as Chiral Building Blocks. In *Chirality in Industry*; Collins, A. N.; Sheldrake, G. N.; Crosby, J. Eds.; John Wiley and Sons, Inc.: New York, 1992; pp. 279-99.
2. Jackson, W. R.; Jacobs, H. A.; Jayatilake, G. S.; Matthews, B. R.; Watson, K. G. *Austr. J. Chem.*, **1990**, 43, 2045-62.
3. Effenberger, F. *Angew. Chem.*, **1994**, 106, 1609-19; *Angew. Chem. Int. Ed. Engl.*, **1994**, 33, 1555-64.
4. Fuhrhop, J.; Penzlin, G. *Organic Synthesis, Concepts, Methods, Starting Materials*, Verlag Chemie, Weinheim, 1983, p. 47.
5. a) Oku, J.; Ito, N.; Inoue, S. *Makromol. Chem.*, **1979**, 180, 1089-91.
   b) Oku, J.; Inoue, S. *J. Chem. Soc., Chem. Comm.*, **1981**, 229-30.
   c) Oku, J.; Ito, N.; Inoue, S. *Makromol. Chem.*, **1982**, 183, 579-86.
6. Asada, S.; Kobayashi, Y.; Inoue, S. *Macromol. Chem.*, **1985**, 186, 1755-62.

7. Matthews, B. R.; Jackson, W. R.; Jayatilake, G. S.; Wilshire, C.; Jacobs, H. A. *Aust. J. Chem.*, **1988**, 41, 1697-709.
8. Minamikawa, H.; Hayakawa, S.; Yamada, T.; Iwasawa, N.; Narasaka, K. *Bull. Chem. Soc. Jpn.*, **1988**, 61, 4379-83.
9. Narasaka, K.; Yamada, T.; Minamikawa, H. *Chem. Lett.*, **1987**, 2073-6.
10. Mori, A.; Ohno, H.; Nitta, H.; Tanaka, K.; Inoue, S. *Synlett*, **1991**, 563-4.
11. Reetz, M. T.; Kunish, F.; Heitmann, P. *Tetrahedron Lett.*, **1986**, 27, 4721-4.
12. Wang, Y.; Chen, S.; Liu, K. K.; Wong, C. *Tetrahedron Lett.*, **1989**, 30, 1917-20.
13. a) Ohta, H.; Kimura, Y.; Sugano, Y. *Tetrahedron Lett.*, **1988**, 29, 6957-60.
    b) Otha, H.; Miyamae, Y.; Tsuchihashi, G. *Agric. Biol. Chem.*, **1989**, 53, 281-3.
    c) Otha, H.; Miyamae, Y.; Tsuchihashi, G. *Agric. Biol. Chem.*, **1989**, 53, 215-22.
    d) Ohta, H.; Kimura, Y.; Sugano, Y.; Sugai, T. *Tetrahedron*, **1989**, 45, 5469-76.
14. Sugai,T.; Ohta, H. *Agric. Biol. Chem.*, **1991**, 55, 293-4.
15. Matsuo, N.; Ohno, N. *Tetrahedron Lett.*, **1985**, 26, 5533-4.
16. van Almsick, A.; Buddrus, J.; Hönicke-Schmidt, P.; Laumen, K.; Schneider, M. P. *J. Chem. Soc, Chem. Comm.*, **1989**, 1391-3.
17. a) Effenberger, F.; Ziegler, T.; Förster, S. *Angew. Chem.*, **1987**, 99, 491-2; *Angew. Chem. Int. Ed. Engl.*, **1987**, 26, 458-60.
    b) Effenberger, F.; Hörsch, B.; Förster, S.; Ziegler, T. *Tetrahedron Lett.*, **1990**, 31, 1249-52.
    c) Effenberger, F.; Hörsch, B.; Weingart, F.; Kühner, S. *Tetrahedron Lett.*, **1991**, 32, 2605-8.
18. a) Brussee, J.; Roos, E. C.; Van der Gen, A. *Tetrahedron Lett.*, **1988**, 29, 4485-8.
    b) Brussee, J.; Loos, W. T.; Kruse, C. G.; Van der Gen, A. *Tetrahedron*, **1990**, 46, 979-86.
19. Niedermeyer, U.; Kula, M. R. *Angew. Chem.*, **1990**, 102, 423-4; *Angew. Chem. Int. Ed. Engl.*, **1990**, 29, 386-7.
20. a) Becker, W.; Benthin, U.; Eschenhof, E.; Pfeil, E. *Biochem. Z.*, **1963**, 337, 156-66.
    b) Becker, W.; Pfeil, E. *J. Am. Chem. Soc.*, **1966**, 88, 4299-300.
21. Wajant, H:; Förster, S.; Böttinger, H.; Effenberger, F.; Pfizenmaier, K. *Plant Science*, **1995**, 108, 1-11.
22. Klempier, N.; Griengl, H.; Hayn, M. *Tetrahedron Lett.*, **1993**, 34, 4769-72.
23. Klempier, N.; Pichler, U.; Griengl, H. *Tetrahedron: Asymmetry*, **1995**, 6, 845-8.
24. Hasslacher, M.; Schall, M.; Hayn, M.; Griengl, H.; Kohlwein, S. D.; Schwab, H. *J. Molec. Biol.*, in print
25. Hörsch, B. *Enzymkatalysierte Synthese von (R)- und (S)-Cyanhydrinen in organischen Lösungsmitteln und deren saure Hydrolyse zu optisch aktiven 2-Hydroxycarbonsäuren*, Universität Stuttgart 1990.
26. Inagaki, M.; Hatanaka, A.; Mimura, M.; Hiratake, J. *Bull. Chem. Soc. Jpn.*, **1992**, 65, 111-20.
27. Ognyanov, V. I.; Datcheva, V. K.; Kyler, K. S. *J. Am. Chem. Soc.*, **1991**, 113, 6992-6.
28. Nitta, H.; Yu, D.; Kudo, M.; Mori, A.; Inoue, S. *J. Am. Chem. Soc.*, **1992**, 114, 7969-75.
29. Ziegler, Th.; Hörsch, B.; Effenberger, F. *Synthesis*, **1990**, 575-8.
30. van Almsick, A. *Esterhydrolasen und Lyasen in der Organischen Chemie: Chirale Cyanhydrine und sekundäre Alkohole sowie deren Ester*, Bergische Universität-GH Wuppertal 1990.
31. Effenberger, F.; Stelzer, U. *Chem. Ber.*, **1993**, 126, 779-86.
32. Schmidt, U.; Gleich, P.; Griesser, H.; Utz, H. *Synthesis*, **1986**, 12, 992-8.
33. Warmerdam, E. G. J. C. *Cyanohydrins in Stereoselective Organic Synthesis*, University of Leiden 1995.

(*Received in Germany* 22 *February* 1996; *accepted* 4 *April* 1996)