Synthesis and Reactions of Optically Active Cyanohydrins

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Cyanohydrins have always held a place of importance both as technical products and as reagents in organic chemistry. It is surprising, therefore, that optically active cyanohydrins have been extensively investigated and employed for syntheses relatively recently. This can be explained by the fact that only in the past few years have enzymatic methods made chiral cyanohydrins readily available in high optical purity. Chiral cyanohydrins are widespread in nature in the form of the respective glycosides and serve roughly 3000 plants and many insects as antifeedants. For the preparative organic chemist, this class of compounds offers an enormous synthetic potential for making other chiral compounds accessible. In a few instances, the pharmacological principle of a drug also incorporates a chiral cyanohydrin as constitutive structural element. In the development of novel, physiologically active compounds all possible stereoisomers must be synthesized and investigated with respect to their activity range and the pathway of their metabolic transformations and/or degradation. The development of simple synthetic procedures for such compounds, which also entail a high degree of stereoselectivity, therefore has prime importance. To this end chiral cyanohydrins may serve as stereochemically pure starting materials.

In the present review, the following topics will be addressed: enantioselective addition of hydrogen cyanide (HCN), catalyzed by the enzymes (R)- and (S)-oxynitrilase, to aldehydes and ketones yielding (R) and (S) cyanohydrins, respectively; enantioselective addition of HCN to aldehydes catalyzed by cyclic dipeptides; enantioselective esterification of racemic cyanohydrins and enantioselective hydrolysis of cyanohydrin esters catalyzed by lipases and esterases, respectively; transformation of the nitrile group of chiral cyanohydrins to provide optically active α-hydroxycarboxylic acids, aldehydes, and ketones, as well as 2-amino alcohols; sulfonylation of the OH group of chiral cyanohydrins to furnish optically active α-sulfonyloxynitriles which undergo S_N2 displacement of the activated OH group yielding α-azido-, α-amino, and α-fluoronitriles with inverted configuration.

1. Introduction

A whole series of procedures has been developed in the last few years for preparing optically active cyanohydrins as precursors for α-hydroxycarboxylic acids, α-hydroxy aldehydes, and vicinal amino alcohols, which have thus become accessible in stereochemically pure form.[1-3]

The stereoselective syntheses of cyanohydrins can be classified into chemical and enzymatic (enzyme-catalyzed) procedures. The most important chemical methods are the diastereoselective addition of trimethylsilyl cyanide[4] and related cyanide-transfer agents[5] to chiral aldehydes, and the enantioselective addition of trimethylsilyl cyanide to aldehydes in the presence of chiral catalysts.[6, 7] Enzymatic procedures leading to optically active cyanohydrins include the oxynitrilase-catalyzed enantioselective addition of HCN to aldehydes, the enantioselective hydrolysis of cyanohydrin ester racemates with es-

terases, and the enantioselective esterification of cyanohydrin racemates with lipases.

In special cases cyclic dipeptides also catalyze the enantioselective addition of HCN to aldehydes;[8] this procedure does not fit into the chemical/enzymatic classification. Formally, cyclic dipeptides can be considered chiral catalysts; however, structural similarities to the active center of oxynitrilases, the structure of which is not yet known, cannot be ruled out completely.

If one weighs the advantages and disadvantages of the chemical and enzymatic methods for preparing optically active cyanohydrins, the scales tip in favor of enzyme-catalyzed processes as general techniques, both with respect to the *ee* values attainable and possible adaptation for the preparation of cyanohydrins on a technical scale. This does not exclude the possibility, though, that other processes might be more advantageous in special cases.

This review focuses on the enzyme-mediated procedures and the use of cyclic dipeptide catalysts for the synthesis of optically active cyanohydrins, in particular aldehyde cyanohydrins, because of their practical importance. In addition, the tremendous potential of optically active cyanohydrins for the synthesis of other important chiral compounds will be discussed.

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2. (R)-Oxynitrilase-Mediated Addition of HCN to Aldehydes

One of the first asymmetric synthesis effected by enzymes ("durch Enzyme bewirkte asymmetrische Synthese"[9]), published by L. Rosenthaler in 1908, concerns the preparation of mandelonitrile from benzaldehyde and HCN, with emulsin as source of the enzymatic catalyst. Rosenthaler gave detailed information on the course of the reaction and the effect of the reaction conditions on the optical yield, but this interesting example of an enzyme-catalyzed asymmetric synthesis was not followed up for many years.[10]

More than fifty years later E. Pfeil et al. took up the idea and developed it into a more general procedure. The enzyme (R)-oxynitrilase [EC 4.1.2.10], which actually catalyzes the addition of HCN to benzaldehyde, was isolated from bitter almonds (*Prunus amygdalus*), purified, and characterized.[11] It was shown that (R)-oxynitrilase accepts other aromatic aldehydes besides its natural substrate benzaldehyde, as well as saturated and unsaturated aliphatic and heterocyclic aldehydes; in each case, the enzyme catalyzes the formation of the corresponding (R) cyanohydrin.[12] (R)-Mandelonitrile was prepared with 86% ee (determined from the specific rotation), which was very high for that time. For less reactive aldehydes, however, the optical yield decreased dramatically; thus, this simple procedure for the preparation of (R) cyanohydrins found virtually no practical application for the next two decades.

All efforts failed to improve the optical yields under the conditions used by Pfeil et al. (solvent: water or water/ethanol; pH 5–6 corresponding to the activity optimum of the enzyme).[12b, 13a] Under these conditions the chemical addition of HCN to aldehydes leading to racemic products cannot be suppressed and prevails especially when the enzyme-catalyzed reaction is slow.

The decisive breakthrough for this process came when it was discovered that the undesirable chemical addition is more or less suppressed in organic solvents that are not miscible with water, for example ethyl acetate or diisopropyl ether (Fig. 1).[13] As Figure 1 shows, the uncatalyzed addition of HCN to benzaldehyde is very slow indeed in ethyl acetate. Thus even for aldehydes that are poor substrates for the enzyme, the enzymatic process predominates over the chemical addition in these media, and products with high ee values result (see Scheme 1, Table 1).[13] In contrast to most other enzyme-catalyzed reac-
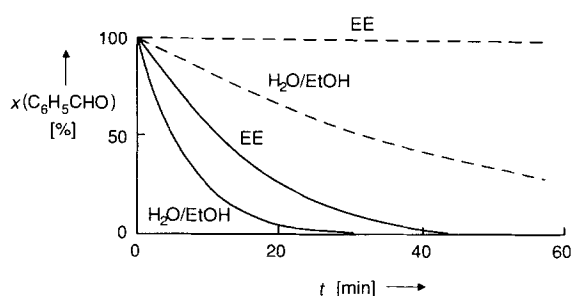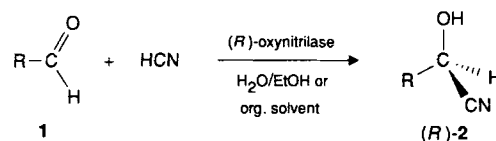

Fig. 1. Comparison of the rate of addition of HCN to benzaldehyde (x = mol fraction) in H₂O/EtOH and in ethyl acetate (EE) without enzyme (- - -) and with enzyme (—).

Scheme 1. Preparation of (R) cyanohydrins by (R)-oxynitrilase-catalyzed addition of HCN to aldehydes.

Table 1. Synthesis of (R) cyanohydrins (R)-2 by enzyme-catalyzed addition of HCN to aldehydes 1 in H₂O/EtOH and in organic solvents [13].

R	in H₂O/EtOH			in EtOAc/Avicel [a]			in iPr₂O/Avicel [a]		
	t [h]	Yield [%]	ee [b] [%]	t [h]	Yield [%]	ee [b] [%]	t [h]	Yield [%]	ee [b] [%]
C₆H₅	1	99	86	2.5	95	99	3	96	>99
3-C₆H₅O-C₆H₄	5	99	11	192	99	98	–	–	
2-furyl	2	86	69	4	88	99	–	–	
3-thienyl	–	--		--	–		6	95	>99
3-pyridyl	2.5	78	7	4.5	89	14	3	97	82
H₃CCH=CH	1.5	68	76	3	68	97	..	–	
H₃CS(CH₂)₂	3	87	60	6.5	97	80	16	98	96
C₃H₇	2	75	69	..	..	–	16	98.6	98
C₆H₅(CH₂)₃	–			–	–		45	94	90
(H₃C)₃C	2.5	56	45	4.5	78	73	4.5	84	83

[a] The enzymes were bound to crystalline cellulose (Avicel). [b] Determined by gas chromatography after either reaction with (R)-α-methoxy-α-trifluoromethyl-phenylacetoyl chloride [(R)-(+)-MTPA chloride] to provide the diastereomeric (R)-(+)-MTPA esters, or after acetylation with acetic anhydride [13].

tions, (R)-oxynitrilase combines low substrate specificity with high enantioselectivity; for example, it accepts aromatic and aliphatic aldehydes and converts them to the respective cyanohydrins with high enantioselectivity (Table 1). Even with sterically demanding substrates such as isopentyl aldehyde, high

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ee values (83%) are obtained. The reaction of 3-phenoxybenz-aldehyde strikingly illustrates the advantage of the organic compared to the aqueous reaction medium: the uncatalyzed reaction is suppressed to the extent that even after 192 h the corresponding (*R*) cyanohydrin is obtained with 98% *ee* (Table 1). Only the reaction of pyridine-3-carbaldehyde gives an unsatisfactory optical yield (14% *ee*) in ethyl acetate. A change of the organic medium to diisopropyl ether suffices to raise the *ee* value once more to a satisfactory 82% (Table 1).

The use of organic solvents in enzyme-catalyzed reactions has been widely reported,[14] especially for such processes where water is the actual reagent. Among these are ester-, amide-, and peptide-forming or -cleaving reactions catalyzed by lipases, amidases, and proteases.[14] Water does not actually take part in the (*R*)-oxynitrilase-catalyzed cyanohydrin formation, but it probably promotes the chemical addition of HCN to the carbonyl double bond.

Of the many organic solvents that have been tested for oxynitrilase-mediated reactions,[15] diisopropyl ether has proven especially advantageous with respect to the *ee* values obtained. This is illustrated strikingly by a number of reactions listed in Table 1 which were carried out in both ethyl acetate and diisopropyl ether. Moreover, enzyme activity in diisopropyl ether can be maintained even over a period of several weeks (Fig. 2). In ethyl acetate, in contrast, (*R*)-oxynitrilase activity is diminished by more than 50% within a few hours (Fig. 2). The lower water content in diisopropyl ether compared to that in ethyl acetate[16] apparently affects both (*R*)-oxynitrilase activity and stability very favorably.
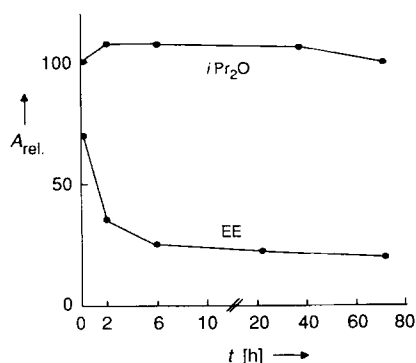

Fig. 2. Plot of the relative activity *A* of (*R*)-oxynitrilase on a crystalline cellulose support (Avicel) in ethyl acetate (EE) and diisopropyl ether (*i*Pr₂O) [15].

For the reactions carried out in organic solvents it is particularly advantageous to employ enzymes bound to a suitable support, for example a crystalline cellulose material such as Avicel. Thus, the "support-bound" enzyme may be filtered off after the reaction is complete and reused as catalyst. (*R*)-Oxynitrilase in organic solvents also accepts rather high substrate concentrations (up to 2 mol L⁻¹) without decreasing the *ee* values; thus, product formation is characterized by a highly favorable space–time yield.[16] The reaction may even be run in a continuous mode: the starting materials are dissolved in the organic solvent and then passed over a column loaded with the support-bound enzyme[15a] or alternatively, kept in a membrane reactor

whence the products are separated from unreacted starting materials and the catalyst by evaporation.[15b]

In aqueous medium the oxynitrilase-catalyzed addition of HCN to aldehydes may be carried out with high selectivity also by simply lowering the pH of the reaction medium below 4.0, where the chemical addition is largely suppressed.[17] Enzyme activity, however, is also reduced at such low pH values. This may be compensated for by employing higher enzyme concentrations; still, reactions with the less reactive aldehyde substrates have poor selectivity. Also, this process is not conductive to the reaction of aldehydes that are poorly or not at all soluble in water.

Many attempts have been reported to avoid the use of the highly toxic, free hydrogen cyanide in the synthesis of cyanohydrins. For instance, aldehydes in water/ethanol have been treated with potassium cyanide and acetic acid in the presence of (*R*)-oxynitrilase; the respective (*R*) cyanohydrins were obtained, though with lower optical yields.[18] Transcyanation with acetone cyanohydrin as HCN donor in the presence of (*R*)-oxynitrilase has been reported to give (*R*) aldehyde cyanohydrins and acetone;[19] however, the selectivities claimed by the authors could not be reproduced.[20]

3. (*S*)-Oxynitrilase-Mediated Addition of HCN to Aldehydes

An enzyme that preferentially cleaves (*S*) cyanohydrins into aldehydes and HCN, and thus may also be expected to catalyze the reverse reaction, was first isolated from *Sorghum bicolor* and characterized by Conn and Bové in 1961.[21a] This (*S*)-oxynitrilase [EC 4.1.2.11] and the (*R*)-oxynitrilase [EC 4.1.2.10] from bitter almonds differ in both structure[11b, 21b, 22] and catalytic properties. The most striking structural difference is that (*R*)-oxynitrilase has a prosthetic group (flavine adenine dinucleotide, FAD) which is missing in (*S*)-oxynitrilase. Removal of the FAD moiety from the (*R*)-oxynitrilase results in complete loss of the catalytic activity.

It is substantially more time-consuming to isolate amounts of (*S*)-oxynitrilase sufficient for preparative purposes from *Sorghum bicolor* than it is to isolate (*R*)-oxynitrilase from bitter almonds. Consequently, this enzyme has been applied in organic syntheses only very recently.[23, 24]

(*S*)-Oxynitrilase from *Sorghum* catalyzes exclusively addition of HCN to aromatic and heteroaromatic aldehydes to yield the respective (*S*) cyanohydrins (Scheme 2), but it does not accept aliphatic aldehydes as substrates.[23, 24] The selectivities in organic solvents are comparable for the two enzymes (Tables 1, 2).

For the synthesis of (*S*) cyanohydrins, reactions in organic solvents rather than in aqueous medium are particularly advantageous since much smaller amounts of the poorly accessible
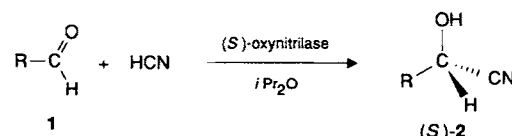
Scheme 2. Preparation of (*S*) cyanohydrins.

Table 2. Synthesis of (S) cyanohydrins (S)-2 by enzyme-catalyzed addition of HCN
to aldehydes 1 in diisopropyl ether [23].

R	(S) Cyanohydrin 2			R	(S) Cyanohydrin 2		
	t [h]	Yield [%]	ee [a] [%]		t [h]	Yield [%]	ee [a] [%]
C₆H₅	3	91	97	3-F₃CC₆H₄	20	87	52
4-ClC₆H₄	48	87	54	3-H₃COC₆H₄	20	93	89
4-H₃CC₆H₄	32	78	87	3-C₆H₅OC₆H₄	144	93	96
3-HOC₆H₄	24	97	91	2-furyl	9	80	80
3-BrC₆H₄	18	94	92	3-thienyl	20 85	97	
3-ClC₆H₄	48	95	91				

[a] Determined by gas chromatography of the (R)-MTPA esters as described in
Table 1.

enzyme are required. In addition some technically interesting
(S) cyanohydrins, such as those from 3-phenoxybenzaldehyde
and various heteroaromatic aldehydes, are difficult to prepare
in an aqueous system owing to the low solubility of the respec-
tive aldehydes.[24]

Recently, the catalytic activity of another (S)-oxynitrilase,
isolated from the leaves of the rubber plant (*Hevea brasiliensis*),
was investigated.[25] In contrast to (S)-oxynitrilase from *Sor-
ghum*, this enzyme was found to accept both aliphatic and aro-
matic aldehydes; however, the enantioselectivity of the reaction
proved to be unsatisfactory for a number of substrates tested.[25]

Despite all improvements in the isolation of (S)-oxynitrilase
from *Sorghum bicolar* L.,[26] industrial application of this en-
zyme requires the respective gene to be cloned in pro- or eucary-
otic organisms and overexpressed. So far, 90% of the native
hydroxynitrile lyase (HNL) has in fact been cloned.[26b]

4. (R)-Oxynitrilase-Mediated Addition of HCN to Ketones

The few optically active ketone cyanohydrins described in the
literature have as a rule been obtained by stereoselective addi-
tion of HCN or of cyanides to ketones that already have a
stereogenic center. Thus, addition of HCN to 17-oxosteroids
proceeds with very high diastereoselectivity.[27] Comparable op-
tical induction is observed for the addition of Me₂Si(CN)₂ to
chiral β-hydroxyketones,[28] of Et₂AlCN to chiral β-ketosulfox-
ides,[29] and of trimethyl-(2-propenyl)silane to chiral acyl
cyanides.[30] The enantioselective hydrolysis of racemic esters of
cyanohydrins with yeast cells from *Pichia miso* gives only poor
chemical yield.[31]

The defense mechanism in which HCN is released from
O-glycosyl cyanohydrins (cyanogenesis) by enzymes, is effective
with both aldehyde and ketone cyanohydrins;[32] thus, ketones
are likewise expected to be substrates for oxynitrilases. For the
(R)-oxynitrilase from bitter almonds [EC 4.1.2.10] the natural
substrate is benzaldehyde; surprisingly, this enzyme also cata-
lyzes the addition of HCN to ketones (Scheme 3),[33] and (R)
cyanohydrins are formed from 2-alkanones in good chemical
yield and with excellent ee values.[33a] 3-Alkanones are much
less suitable substrates; the respective cyanohydrins are ob-
tained only with unsatisfactory chemical yield and also much
lower optical purity (Table 3).[33] The best results for the synthe-
sis of (R) ketone cyanohydrins are again obtained in organic

Scheme 3. Preparation of (R) ketone cyanohydrins.
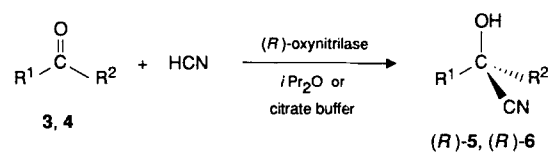
3, 5 : R¹ = alkyl, R² = CH₃
4, 6 : R¹ = alkyl, R² = C₂H₅

Table 3. Synthesis of (R) ketone cyanohydrins (R)-5 (R² = CH₃) and (R)-6
(R² = C₂H₅) by enzyme-catalyzed addition of HCN to ketones 3 (R² = CH₃) and
4 (R² = C₂H₅), respectively, in diisopropyl ether [33a] and in aqueous citrate buffer
[33b].

R¹	in iPr₂O/Avicel [a]				in citrate buffer [b]	
	(R)-5 [c]		(R)-6 [d]		(R)-5 [c]	
	Yield [%]	ee [%]	Yield [%]	ee [%]	Yield [%]	ee [%]
nC₃H₇	70	97	33	85	78	95
nC₄H₉	90	98	21	90	94	98
nC₅H₁₁	88	98	7	66	56	96
(H₃C)₂CHCH₂	57	98	–	–	40	98

[a] 20 U enzyme per mmol ketone. [b] 50 U enzyme per mmol ketone.
[c] Enantiomeric excesses of the diastereomeric (R)- or (S)-MTPA esters deter-
mined by gas chromatography [33]. [d] Enantiomeric excesses determined by gas
chromatography on β-cyclodextrin columns after saponification to give the corre-
sponding carboxylic acids and esterification with diazomethane [33b].

solvents; the ee values for reactions in citrate buffer (pH ≤ 4)
are comparable if at least double the amount of enzyme is em-
ployed (Table 3).[33b] (S)-Oxynitrilase from *Sorghum bicolor*
does not accept ketones as substrates.[24]

5. Cyclodipeptides as Catalysts for the Enantioselective Addition of HCN to Aldehydes

Inoue et al. showed that cyclic dipeptides (1,4-dioxopiper-
azines) containing a histidine moiety also catalyze the asymmet-
ric addition of HCN to benzaldehyde to provide optically active
mandelonitrile.[8, 34] The highest selectivity (87% ee of (R)-
mandelonitrile) was obtained with the cyclic dipeptide from (S)-
histidine and (S)-phenylalanine (Table 4).[34c] The reaction is
not restricted to benzaldehyde. It has been extended to other
aromatic aldehydes and numerous heterocyclic and aliphatic
aldehydes; in most cases, though, the selectivity was unsatisfac-
tory (Table 5).[34c, 35, 36] With ketones, the corresponding
cyanohydrins are obtained with very low enantioselectivi-

Table 4. Cyclic dipeptides as catalysts for the enantioselective addition of HCN to
benzaldehyde.

Catalyst	Mandelonitrile			Ref.
	Yield [%]	Config.	ee [%]	
cyclo[(S)-Ala-(S)-His]	50	R	9.9	[8a, 34a]
cyclo[(R)-Ala-(S)-His]	90	R	7.5	[8a, 34a]
cyclo[(S)-His-(S)-His]	50	R	2.5	[8a, 34a]
cyclo[(S)-His-(S)-Leu]	85	S	55	[34b]
cyclo[(S)-His-(S)-Phe]	40	R	90	[8b]
cyclo[(S)-His-(S)-Phe]	97	R	97	[34c]
cyclo[(R)-His-(R)-Phe]	86	S	79	[36a]

Table 5. Enantioselective addition of HCN to aldehydes 1 catalyzed by cyclo[(S)-His-(S)-Phe] to give (R) cyanohydrins (R)-2.

R	(R)-2 Yield [%]	ee [%]	Ref.	R	(R)-2 Yield [%]	ee [%]	Ref.
3-C6H5OC6H4	97	92	[34c]	4-F3CC6H4	90	80	[36b]
2-H3COC6H4	45	84	[34c]	4-(CH3)2NC6H4	50	53	[36b]
3-H3COC6H4	97	90	[36b]	2-naphthyl	100	86	[36b]
4-H3COC6H4	85	83	[36b]	2-furyl	76	93	[36b]
4-H3CC6H4	91	92	[36b]	2-pyrrolyl	31	0	[36b]
4-O2NC6H4	77	29	[36b]	2-thienyl	70	58	[36b]
3-HOC6H4	75	67	[35b]	3-pyridyl	89	0	[36b]
4-HOC6H4	86	35	[36b]	C3H7	100	26	[36b]
4-ClC6H4	96	66	[36b]	C6H5CH2	100	14	[36b]
4-NCC6H4	100	32	[34c]	(H3C)2CH	79	71	[34c]
4-C6H5OC6H4	100	81	[36b]	c-C6H11	96	58	[34c]

ties.[36b] In virtually all cases examined so far, cyclic dipeptide catalysts with (S,S) configuration favor the formation of (R) cyanohydrins. Only with cyclo[(S)-His-(S)-Leu] are (S) cyanohydrins formed preferentially.[34b] The dipeptide catalyst cyclo[(R)-His-(R)-Phe] gives an excess of the (S) product, the respective cyclo[(S)-His-(S)-Phe] an excess of the (R) cyano-hydrin (Table 4).[36a] This possibility of inverting the product configuration by simply changing the catalyst was employed in the synthesis of (S)-3-phenoxybenzaldehyde cyanohy-drin,[36, 37] the alcohol component of a commercially important pyrethroid.[38]

The mechanism of the enantioselective addition of HCN to aldehydes under cyclic dipeptide catalysis still remains to be unraveled. Nothing definite is known yet, for instance, of the structure of the transition state or of intermediates, despite many efforts.[36a, 39]

Acyclic dipeptides also show limited catalytic activity for enantioselective HCN addition to aldehydes; however, accept-able selectivities were obtained only in the presence of certain titanium complexes.[40]

6. Preparation of (R) and (S) Cyanohydrins by Kinetic Resolution with Esterases and Lipases

Aldehyde cyanohydrins are secondary alcohols and thus may form esters with carboxylic acids. Esterase-catalyzed hydrolysis or lipase-catalyzed esterification thus might be utilized to sepa-rate the enantiomers of either racemic cyanohydrins or cyanohydrin esters.[41]

6.1. Enantioselective Hydrolysis of Racemic Cyanohydrin Esters

If saponification of cyanohydrin acetate racemates catalyzed by ester hydrolases from *Pseudomonas sp.* is carried out in a phosphate buffer at pH 7, only the unreacted cyanohydrin ac-etates are isolated in stereochemically pure form. Under the reaction conditions the chiral cyanohydrins apparently are racemized.[42] Only in a few cases are the free cyanohydrins isolated as more or less pure enantiomers.[43] Hydrolysis with lipases in a sodium acetate buffer at pH 4.5 without exception

affords both cyanohydrin esters and cyanohydrins in optically active form.[41] Lipases from *Pseudomonas fluorescens* (A), lipase PS (B) and lipase P (C), preferentially cleave the (S) cyanohydrin acetates; with lipases from *Candida cylindracea* (D) and with lipase AY (E), on the other hand, the (R) cyanohydrins are formed (Scheme 4).[41] For the lipases A, B, and C, aromatic
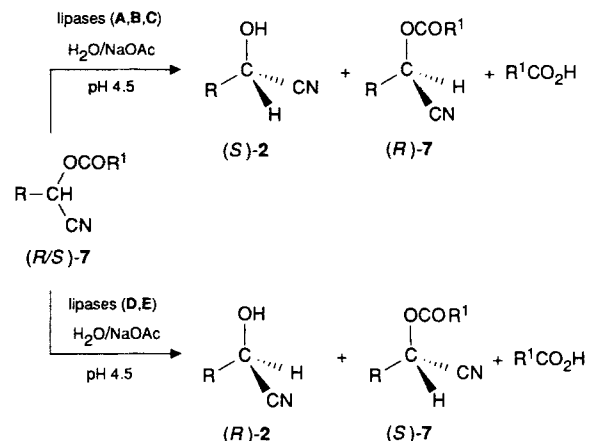
Scheme 4. Enantioselective hydrolysis of racemic cyanohydrin esters (R = C3H7, C6H5; R¹ = CH3, C3H7, C5H11, C6H5CH2) with various lipases to give (R) cyanohydrins and (S) cyanohydrin esters, and (S) cyanohydrins and (R) cyanohy-drin esters; lipase from *Pseudomonas fluorescens* (A), lipase PS (B), lipase P (C), lipase from *Candida cylindracea* (D), and lipase AY (E).

cyanohydrin esters are good substrates (R = C6H5), but aliphatic derivatives (R = C3H7) are not. Butyrates (R¹ = C3H7) yield (S) cyanohydrins with the highest selectivities (97% ee). The situation is reversed for lipases D and E; for these enzymes, the aliphatic cyanohydrin esters (R = C3H7) are good substrates, and the butyrates (R¹ = C3H7) once again give (R) cyanohydrins with the highest selectivities (up to 86% ee).

6.2. Enantioselective Esterification of Cyanohydrin Racemates

Chiral cyanohydrins do not racemize to any significant extent in organic solvents. Lipase-catalyzed enantioselective esterifica-tion of a cyanohydrin racemate in organic solvents thus appears especially suitable for racemate resolution when the unprotected optically active cyanohydrin is desired.[41, 43] With vinyl acetate, for which acylation is not reversible,[44] and in dichloromethane as solvent,[41] the lipases A-D exhibit the same configurational specificity as that observed in the enantioselective hydrolysis of the ester racemates described in Section 6.1 (Scheme 5).

The highest optical yields for the (S) acetates (S)-7, up to 98% ee, for aromatic cyanohydrins have been obtained with *Pseudomonas fluorescens* lipase (A),[41] which also catalyzes the acetylation of aliphatic cyanohydrins yielding the acetates (S)-7.

One principal disadvantage of kinetic resolution is that at most 50% of the racemic material can be obtained as the desired enantiomer. It is generally necessary, therefore, to subsequently racemize the remaining undesired enantiomer and to return the resulting racemate to the process. The enantioselective acetyla-tion of a cyanohydrin racemate may also be carried out with
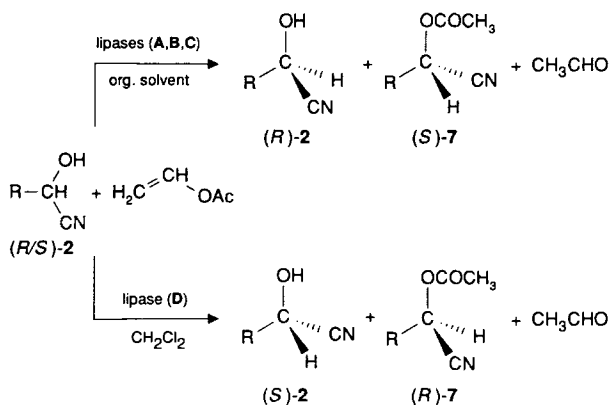
Scheme 5. Enantioselective esterification of cyanohydrin racemates (R = C₃H₇, C₅H₅, C₆H₅CH₂CH₂, 4-HOC₆H₄, 3-CH₃OC₆H₄, etc.) with vinyl acetate in organic solvents with various lipases (cf. Scheme 4).

isopropenyl acetate in an organic solvent and in the presence of a basic ion exchange resin that is sufficiently basic to cause racemization of the cyanohydrin, but not of the optically active cyanohydrin acetate formed.[45] Thus, a subsequent, separate racemization is no longer required, and the racemic substrate can be completely converted into one enantiomer. The subsequent hydrolysis of the enantiomerically pure cyanohydrin acetates can be conducted under nonracemizing conditions.

Apart from the enantioselective hydrolysis of cyanohydrin ester racemates (Section 6.1) and the enantioselective esterification of such racemic cyanohydrins (Section 6.2), racemic mixtures of cyanohydrin esters can be resolved by lipase-catalyzed transesterification with high-boiling alcohols in organic solvents.[41, 46]

7. Stereoselective Reactions of Optically Active Cyanohydrins

Since chiral cyanohydrins as α-substituted carboxylic acid derivatives have considerable synthetic potential, subsequent stereoselective reactions should lead to other important classes of compounds with stereogenic centers. Two types of reactions of cyanohydrins can be differentiated: reactions of the CN function which do not involve the α-hydroxyl moiety, and those in which reaction occurs at the OH group. Application of either reaction type in general synthesis requires racemization-free processes.

7.1. Reactions of the Nitrile Group

In contrast to α-amino acids, only a few optically active α-hydroxycarboxylic acids are found in nature. General processes for the preparation of chiral α-hydroxycarboxylic acids therefore had to be developed. Today, they are accessible by straightforward chemical synthesis,[47] by fermentation,[48] and by enzymatic processes.[49, 50] Specific (S) α-hydroxycarboxylic acids may be obtained by diazotization of natural L α-amino acids in aqueous medium. The diazonium salts formed initially undergo intramolecular substitution to the respective α-lactones

with inversion of configuration; these are hydrolyzed, again with inversion, to give α-hydroxycarboxylic acids with a configuration identical to that of the starting α-amino acid. The (S) enantiomers thus obtained may be converted into the corresponding (R)-2-hydroxycarboxylic acids by activation of the OH group followed by S_N2 displacement with an oxygen nucleophile.[51]

The racemization-free hydrolysis of chiral cyanohydrins offers an interesting general route to (R) and (S) 2-hydroxycarboxylic acids. Hydrolysis of an optically active cyanohydrin with concentrated hydrochloric acid was first described for mandelonitrile where it was used to probe for the optical purity of the nitrile.[10a] Other authors later on assumed that in some cases acid-catalyzed hydrolysis of unprotected cyanohydrins resulted in partial racemization.[31, 35a] O-protected cyanohydrins, on the other hand, are hydrolyzed with a high degree of retention of configuration to the respective α-hydroxycarboxylic acids in both acidic[52] and basic media.[44a] Since both the formation of a cyanohydrin and its cleavage to the aldehyde and HCN are base catalyzed, one might expect that the acid-catalyzed hydrolysis of unprotected cyanohydrins would proceed without racemization.

(R) and (S) cyanohydrins derived from aldehydes[13c, 23] and (R) ketone cyanohydrins[33] are indeed hydrolyzed by concentrated hydrochloric acid to give the corresponding (R) and (S) hydroxycarboxylic acids, respectively, in excellent chemical yield and with complete retention of configuration (Schemes 6, 7; Tables 6, 7). Actually, such hydrolyses can be carried out quite simply. In the first step, the optically active cyanohydrins are prepared from the parent carbonyl compound and HCN in an organic solvent under enzyme catalysis. The Avicel-bound enzyme is filtered off, the solvent removed, and the cyanohydrin residue hydrolyzed with concentrated hydrochloric acid directly and without any additional purification step. The HCl is removed, and the α-hydroxycarboxylic acids are extracted with
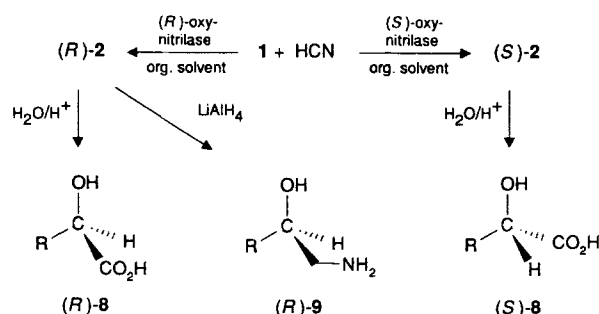
Scheme 6. Acid-catalyzed hydrolysis of (R) and (S) cyanohydrins to give (R) and (S) 2-hydroxycarboxylic acids, respectively, and racemization-free hydrogenation of (R) cyanohydrins to give (R) 2-amino alcohols.

(R)-10 : R¹ = alkyl, R² = CH₃
(R)-11 : R¹ = alkyl, R² = C₂H₅
(R)-10, (R)-11
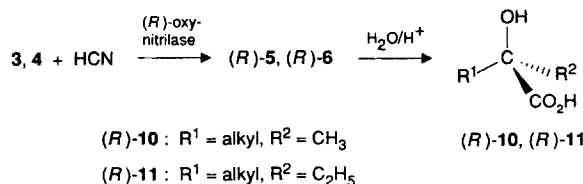
Scheme 7. Acid-catalyzed hydrolysis of (R) ketone cyanohydrins to give (R) 2-hydroxycarboxylic acids.

Table 6. Acid-catalyzed hydrolysis of the (R) and (S) cyanohydrins **2** [a] (prepared in situ by enzyme-catalyzed addition of HCN to aldehydes **1**) to give (R) and (S) α-hydroxycarboxylic acids **8**, respectively.

R	(R)-2 ee [%]	(S)-2 ee [%]	(R)-8 Yield [%]	(R)-8 ee [b] [%]	(S)-8 Yield [%]	(S)-8 ee [b] [%]
C_6H_5	99	97	82	99	77	98
4-$H_3CC_6H_4$	98	78	82	98	75	78
4-ClC_6H_4	99	54	71	99	63	54
3-$C_6H_5OC_6H_4$	97	96	72	97	70	96
3-$O_2NC_6H_4$	89	–	81	87	–	–
$C_6H_5CH_2$	74	–	90	74	–	–
c-C_6H_{11}	99		88	97		–
3-BrC_6H_4	–	92	–	–	76	90
3-ClC_6H_4	–	91	–		69	91
3-$H_3COC_6H_4$	–	89	–	–	67	90
$H_3C(CH_3)_2$	96	–	85	96	–	
2-$H_3CS(CH_2)_2$	98		87	98		–

[a] Crude product. [b] Determined by gas chromatography after formation of the isopropyl ester (treatment with HCl-saturated isopropanol) and subsequent derivatization to the diastereomeric (R)-MTPA esters [13, 23].

Table 7. Acid-catalyzed hydrolysis of the (R) ketone cyanohydrins (R)-5 and (R)-6 (prepared in situ by enzyme-catalyzed addition of HCN to ketones **3** and **4**, respectively) to α-hydroxycarboxylic acids (R)-10 and (R)-11, respectively [33].

R^1	R^2	(R)-5, (R)-6 ee [%]	(R)-10, (R)-11 Yield [%]	ee [a] [%]
C_2H_5	CH_3	76	85	76
nC_3H_7	CH_3	97	64	99
nC_4H_9	CH_3	98	72	98
nC_5H_{11}	CH_3	98	78	98
$(H_3C)_2CH$	CH_3	90	76	90
$(H_3C)_2CHCH_2$	CH_3	98	89	96
$(H_3C)_2CH(CH_2)_2$	CH_3	98	88	99
nC_3H_7	C_2H_5	85	82	85
nC_4H_9	C_2H_5	90	89	90

[a] Determined by gas chromatography on β-cyclodextrin columns after esterification with diazomethane [33].

diethyl ether, isolated, and characterized. Comparison of the optical purity of the crude cyanohydrins with that of the isolated and purified α-hydroxycarboxylic acids shows hydrolysis to proceed virtually without racemization in all cases investigated (Tables 6, 7).

A wide spectrum of cyanohydrins is easily accessible by the enzymatic procedure outlined above. The acid-catalyzed hydrolysis of the optically active cyanohydrins, which can be conducted without racemization, thus represents a general route for the preparation of chiral α-hydroxycarboxylic acids.

Optically active α-hydroxy aldehydes are also important structural elements in natural products and thus might also be employed as chiral starting materials in asymmetric syntheses.[52] Direct conversion of optically active cyanohydrins into the corresponding α-hydroxy aldehydes would make this class of compounds easily accessible. Cyanohydrin racemates in fact have been hydrogenated with Raney nickel in acidic medium to α-hydroxy aldehydes.[53a] When this procedure was extended to optically active cyanohydrins, the configuration of the isolated products indicated that hydrogenation occurs with a high degree of retention of configuration.[53b] Because of the moderate yields and the difficult product isolation, however, selective hydrogenation of α-hydroxycarboxylic acids (see above) appears

a much more favorable route to chiral α-hydroxy aldehydes.[52d, 54]

Hydrogenation of chiral cyanohydrins to 2-amino alcohols is also of considerable preparative interest, since these compounds have a wide spectrum of biological activity.[55] They may be categorized as adrenalin-type derivatives (amino function at a primary carbon atom) or ephedrin-type derivatives (amino function at a secondary carbon atom). Adrenalin-type 2-amino alcohols can be prepared from O-protected chiral cyanohydrins by various hydrogenating procedures without any trace of racemization.[4a, 43a, 56] Interestingly, free chiral cyanohydrins may also be hydrogenated directly and without racemization to the corresponding amino alcohols.[13c] Here, as in the synthesis of α-hydroxycarboxylic acids, the chiral cyanohydrins obtained from enzyme-catalyzed HCN addition in organic solvents can be applied directly, without any specific purification, for the hydrogenation process (Scheme 6, Table 8).[13c]

Table 8. Hydrogenation of (R) cyanohydrins (R)-2 (prepared in situ by enzyme-catalyzed addition of HCN to aldehydes **1**) with $LiAlH_4$ to give amino alcohols (R)-9 [13c].

R	(R)-2 ee [%]	(R)-9 Yield [%]	(R)-9 ee [a] [%]	R	(R)-2 ee [%]	(R)-9 Yield [%]	(R)-9 ee [a] [%]
C_6H_5	99	92	99	2-$H_3CS(CH_2)_2$	98	31	98
3-$C_6H_5OC_6H_4$	98	97	95	nC_3H_7	96	99	89
4-$H_3CC_6H_4$	99	100	>98	2-furyl	98	71	98
c-C_6H_{11}	91	94	80	$(H_3C)_3C$	93	70	>90

[a] Determined by gas chromatography on Chiracil-Val columns after conversion into the corresponding (R)-β-N,O-bis(trifluoroacetyl)amino alcohols by treatment with trifluoroacetic acid [13c].

Preparation of 2-amino alcohols of the ephedrin type starting from cyanohydrins was first described by Krepski et al.; O-protected racemic cyanohydrins were reacted with Grignard reagents, and the resulting imino intermediates were subsequently hydrogenated with $NaBH_4$.[57] This method was also applied successfully to (R) and (S) cyanohydrins.[58, 59] Investigation of the stereochemistry of this reaction for both (R) and (S) cyanohydrins established that the Grignard addition pro-
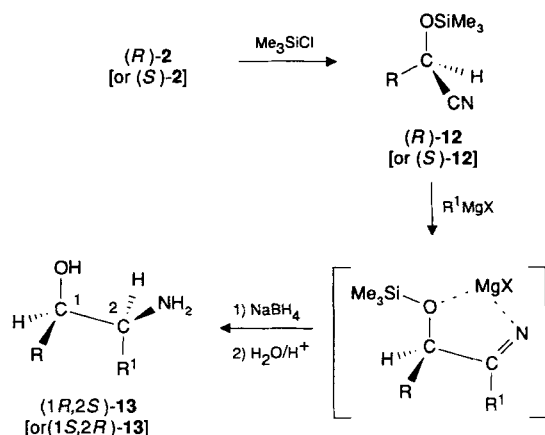
Scheme 8. Stereoselective synthesis of 2-amino alcohols. R = C_3H_7, C_6H_5; R^1 = CH_3, C_2H_5, C_6H_5; X = Br, I.

ceeds without any racemization at C-1. The hydrogenation at C-2 is highly diastereoselective owing to chelate control; *erythro* products are formed almost exclusively (Scheme 8, Table 9).[59] On the other hand, the *threo* compounds are formed predominantly when *N*-diisobutylaluminum hydride is added to the O-protected cyanohydrin before the organolithium reagent is added.[60] This procedure, however, is decidedly less diastereoselective.[60]

Table 9. Synthesis of 2-amino alcohols (1 R,2S)- and (1S,2R)-13 from the respective cyanohydrins (R)- and (S)-2 (crude products) by formation of the trimethylsilyl ethers (R)- and (S)-12, Grignard reaction, and subsequent hydrogenation of sodium borohydride [59].

| R | Cyanohydrin 2 | | 2-Amino alcohol (1 R,2S)- or (1S,2R)-13 | | | | | |
	ee [%]	Config.	R¹	Yield [a] [%]	*ee* [b] [%]	*de* [b] [%]	*ee* [c] [%]	*de* [c] [%]
C₆H₅	>99	(R)	C₆H₅	83	99	99	100	100
C₆H₅	>99	(R)	C₂H₅	53	97	94	>99	>99
C₆H₅	>99	(R)	CH₃	50	99	92	99	>99
C₃H₇	95	(R)	C₆H₅	93	92	93	>99	>99
C₃H₇	95	(R)	CH₃	44	93	63	–	63
C₆H₅	>99	(S)	C₆H₅	95	99	99	100	100
C₆H₅	>99	(S)	CH₃	66	99	90	>99	>99

[a] As the hydrochloride. [b] Determined by gas chromatography after treatment of the crude products with pivaloyl chloride and filtration through silica gel. [c] Determined after one recrystallization.

In both hydrogenation[61a] and Grignard reaction[61b] with O-protected cyanohydrins, imines are formed initially; these may be subjected to transimination with primary amines to furnish biologically interesting N-substituted amino alcohols. Addition of HCN to the imino compounds affords β-hydroxy α-amino acids stereoselectively, if one starts out with a chiral cyanohydrin.[62] Hydrolysis of the imino compounds formed by addition of Grignard reagents to O-protected chiral cyanohydrins yields optically active acyloins.[18a, 63]

7.2. Reactions of the Hydroxyl Group

The synthetic potential of optically active cyanohydrins can be extended tremendously if the OH group is converted into a good leaving group which may be exchanged stereoselectively with a wide range of nucleophiles.[64] Nucleophilic substitution reactions with activated α-hydroxycarboxylic acids and esters are well established,[51a, 65] but little is known about the analogous reactions of α-hydroxynitriles.[66] So far only α-halonitriles have been prepared in optically pure form.[66] The halide ion released upon reaction of these compounds is a good nucleophile and leads to partial racemization of the chiral α-halonitriles during nucleophilic substitution.[66] α-Sulfonyl-oxynitriles (R)-14, which are easily accessible from chiral cyanohydrins (R)-2 by sulfonylation, have a much higher configurational stability than the α-halonitriles (Scheme 9).[64] α-Sulfonyloxynitriles derived from aliphatic cyanohydrins are considerably more stable than those from aromatic cyano-hydrins.[64] Under mild conditions aliphatic α-sulfonyloxyni-triles (R)-14 react with nucleophiles with complete inversion of configuration; the aromatic analogues react with weak nucleo-philes with at least partial racemization.[64]

Nucleophilic substitution of optically active α-sulfonyloxy-nitriles (R)-14 allows access to a great variety of interesting compounds, such as α-azidonitriles (S)-15, α-aminonitriles (S)-16, α-amino acids (S)-17, and aziridines (S)-18 in optically pure form (Scheme 9).[64] Aliphatic cyanohydrins (S)-2, which are not accessible by the (S)-oxynitrilase-catalyzed addition of HCN to aldehydes,[23, 24] may be prepared by S_N2 reactions of the stable (R) 2-sulfonyloxynitriles (R)-14 with acetates.[64b] The resulting cyanohydrin esters (S)-19 then are hydrolyzed to the cyanohydrins (S)-2.
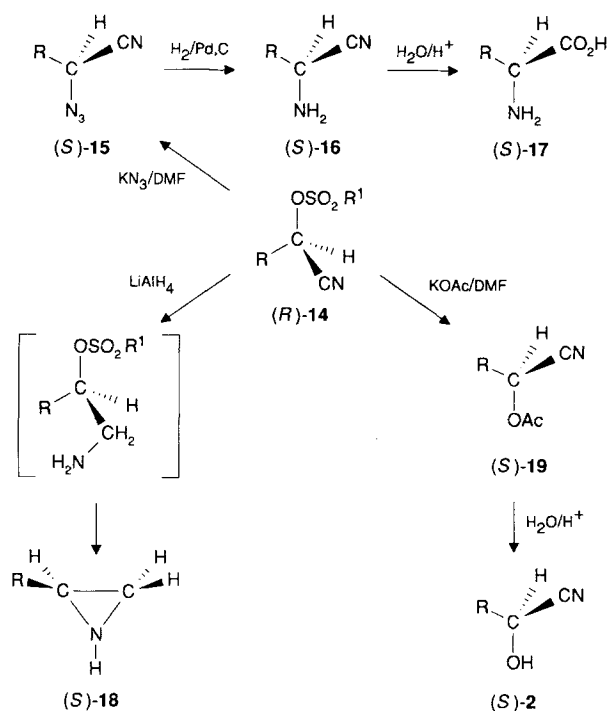
Scheme 9. Stereoselective reactions of sulfonyl-activated (R) cyanohydrins with nucleophiles with inversion of configuration. R¹ = CH₃, CF₃, 4-CH₃C₆H₄.

Sulfonyl-activated cyanohydrins are often employed in synthesis, since both the cyanohydrin and the nucleophile can be varied. In addition, direct stereoselective reactions of the cyanohydrin OH group are also feasible with special reagents. (S) Fluoronitriles (S)-20, for instance, may be prepared directly and in good optical yield from the (R) cyanohydrins (R)-2 by reaction with diethylaminosulfur trifluoride (DAST).[67] These α-fluoronitriles (S)-20 then are hydrolyzed to the α-fluoro-carboxylic acids (S)-21 without notable racemization (Scheme 10).[67]
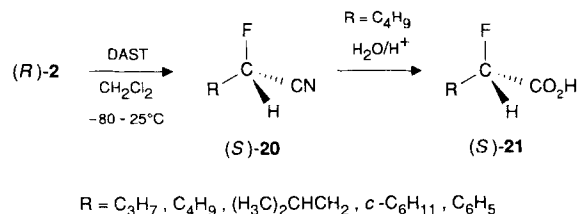
R = C₃H₇, C₄H₉, (H₃C)₂CHCH₂, c-C₆H₁₁, C₆H₅

Scheme 10. Preparation of (S) 2-fluoronitriles and (S)-2-fluorocarboxylic acids from (R) cyanohydrins.

Another example of a stereoselective reaction in this context is the Mitsunobu reaction of optically active cyanohydrins in the presence of carboxylic acids, which takes place with inversion of configuration, thus permitting preparation of (S) cyanohydrins directly from their (R) isomers.[68]

8. Summary and Outlook

The enzyme-catalyzed asymmetric synthesis of cyanohydrins known for close to ninety years has only in the last decade turned into an important instrument for organic synthesis. The markedly improved enantioselectivities are possible primarily by suppression of the chemical cyanohydrin formation. This can be achieved either by employing an organic solvent or by working at low pH values.

(R) Cyanohydrins in particular have become easily accessible by this procedure, and their considerable synthetic potential as α-substituted carboxylic acid derivatives has been firmly established within a few years. Reactions of optically active cyanohydrins can be conducted with retention of chirality, and further stereogenic centers can be introduced diastereoselectively. Thus chiral cyanohydrins open up routes to other important classes of compounds with stereogenic centers. The synthetic potential of chiral cyanohydrins appears particularly important for the diastereoselective synthesis of drugs with stereogenic centers. Even if drugs like 2-amino alcohols are still applied in racemic form today, all stereoisomers are required for registration, since the activity spectrum and metabolism of each isolated stereoisomer must be established. Chiral cyanohydrins frequently open simple and direct routes to the individual stereoisomers.

The (R)-oxynitrilase from bitter almonds has a broad substrate spectrum and can be obtained readily in industrial quantities. In contrast, the substrate spectrum of (S)-oxynitrilase from Sorghum is restricted mainly to aromatic aldehydes, and the poor availability of this enzyme is more or less prohibitive for industrial applications. Future developments in the synthesis of optically active cyanohydrins are thus expected primarily in extending the substrate spectrum of (S)-oxynitrilases and in improving their availability. (S)-oxynitrilases from other organisms (plants, insects) may extend and improve the substrate spectrum. The poor accessibility of (S)-oxynitrilases, however, will be overcome only by cloning and overexpression in a sufficiently efficient production organism.

Modification of the active center(s) of the oxynitrilases, which is prerequisite for further optimization of the substrate selectivity, requires knowledge of the complete enzyme structure. Currently, little or rather almost nothing is known about the three-dimensional structure of oxynitrilases. If this deficit can be overcome, oxynitrilases will gain even more importance in the synthesis of chiral compounds.

The enthusiastic commitment of my co-workers, who are cited in the references, has helped to bridge the gap between organic synthesis and enzymology which has been decisive for our successful exploitation of chiral cyanohydrins in synthesis. My special gratitude is due to Drs. Thomas Ziegler and Siegfried Förster who played a major role from the beginning in the development of the work described in this review. Dr. Angelika Baro was strongly

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