(R)- and (S)-Cyanohydrins - Their Enzymatic Synthesis and Their Reactions

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ABSTRACT. The known enzymes (R)- and (S)-oxynitrilase catalyze the enantioselective addition of hydrocyanic acid to aldehydes to give (R)- and (S)-cyanohydrins. The optical yields can distinctly be improved by the application of organic solvents (i.e. ethyl acetate or diisopropyl ether) instead of a water/ethanol mixture which was used previously in these reactions. For the enzyme (S)-oxynitrilase Sorghum bicolor evolved to be the best source.
The optically active cyanohydrins can be transformed without any racemization by acid catalyzed hydrolysis into α-hydroxy acids and by hydrogenation with lithium/aluminum hydride into 1,2-amino alcohols. Via addition of Grignard reagents to the O-protected cyanohydrins and follow-up hydrogenation, 1,2-amino alcohols are gained with very high diastereoselectivity. By O-sulfonylation of the (R)- and (S)-cyanohydrins optically active α-sulfonyloxy nitriles are obtained. These nitriles react with various nucleophiles by complete inversion of configuration to form various α-substituted carboxylic acid derivatives, α-azido nitriles, α-amino nitriles, α-amino acids, etc.

Enzyme Catalyzed Preparation of (R)-Cyanohydrins

E. Pfeil et al. have investigated the (R)-oxynitrilase (E.C. 4.1.2.10) catalyzed addition of hydrocyanic acid to various aldehydes to give (R)-cyanohydrins [1]. In the applied solvents, water or water/alcohol mixtures, the normal chemical addition of HCN cannot be avoided which results in pure optical yields in most cases. All efforts to improve the optical yields in water/alcohol solutions failed. By using organic solvents, which are not miscible with water, however, the (R)-cyanohydrins are obtained in excellent chemical and optical yields [2].

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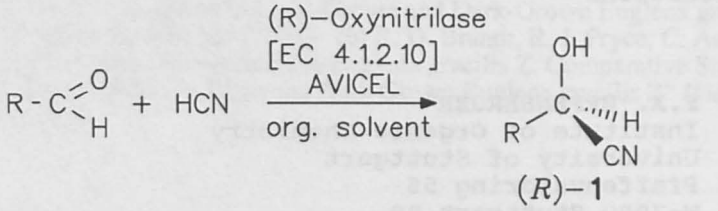
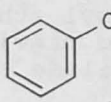
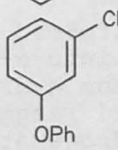
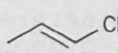
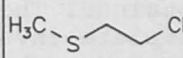
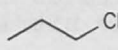
Although the substrate specificity of the enzyme is fairly low - structurally very different aliphatic as well as aromatic aldehydes are good substrates - the enantioselectivity of (R)-oxynitrilase is very high.



(*R*)–**1**

Aldehydes	H_2O/EtOH Time [h]	Yield [%]	ee[a] [%]	EE/Cellulose[b] Time [h]	Yield [%]	ee[a] [%]
	1	99	86	2.5	95	99
	5	99	10.5	192	99	98
	1.5	68	76	3	68	97
	3	87	60	6.5	97	80
	2	75	69	4.5	75	96
	2.5	56	45	4.5	78	73

[a] The optical purity of the cyanohydrins was determined via the diastereomeric esters with (*R*)– α–methoxy–α–trifluoromethyl–phenylacetyl chloride [(R)–(+)–MTPA chloride] by gas chromatography

[b] EE = ethyl acetate

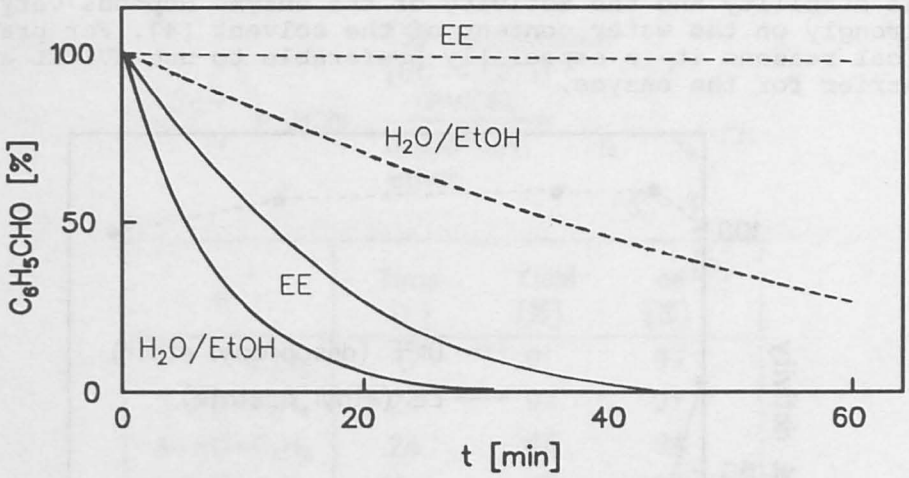
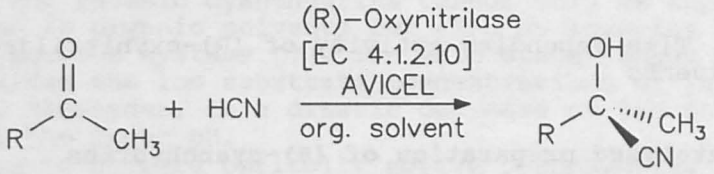


Figure 1. Rate of the chemical (---) and enzymatic (——) addition of HCN to benzaldehyde (initial concentration 5×10^{-3} M) in H_2O/EtOH and in ethyl acetate (EE)/cellulose.

(R)-Oxynitrilase also catalyzes the enantioselective addition of hydrocyanic acid to ketones. In organic solvents optically active ketone cyano hydrins, which are important intermediates for the preparation of the respective hydroxy carboxylic acids, are obtained with high optical purities [3].



R	Yield [%]	ee [%]
CH_3CH_2	80	76
$CH_3CH_2CH_2$	90	97
$CH_3CH(CH_3)-CH_2$	57	98
$H_2C=CH-CH_2-CH_2$	80	97

The stability and the activity of the enzyme depends very strongly on the water content of the solvent [4]. For practical reasons it is especially preferable to use AVICEL as a carrier for the enzyme.

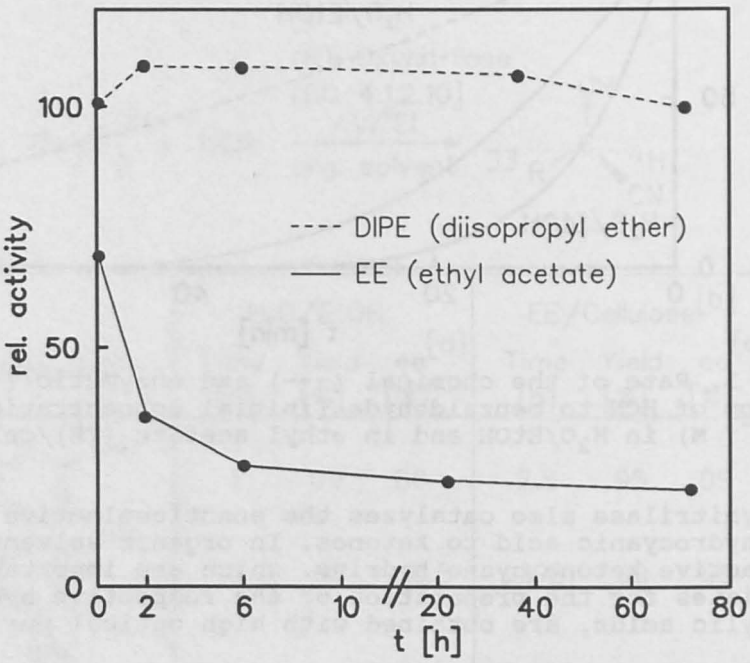
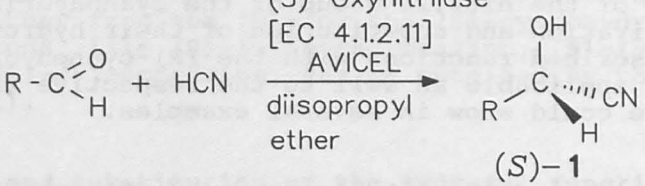


Figure 2. Time depending activity of (R)-oxynitrilase in organic solvents

Enzyme catalyzed preparation of (S)-cyanohydrins

The enantioselective addition of HCN to aldehydes to give (S)-cyanohydrins would be a simple entry to important (S)-α-substituted carboxylic acid derivatives. Therefore, we have investigated the (S)-oxynitrilase (E.C. 4.1.2.11) catalyzed preparation of (S)-cyanohydrins. In contrary to the (R)-enzyme the (S)-oxynitrilase is not available commercially [5]. After an extensive search, sprouts of Sorghum bicolor have proved to be the best source for (S)-oxynitrilase.
The application of the (S)-enzyme for the preparation of (S)-cyanohydrins was performed in full analogy to the application of the (R)-oxynitrilase in organic solvents. For (S)-oxynitrilase only aromatic aldehydes are substrates. The optical yields obtained are comparable to the reactions with (R)-oxynitrilase [6].



(*S*)–**1**

R	Time [h]	Yield [%]	ee[a] [%]
C_6H_5	3	91	97
3–HO–C_6H_4	24	97	91
4–HO–C_6H_4	24	84	94
3–PhO–C_6H_4	144	93	96
3–Br–C_6H_4	18	94	92
4–CH_3–C_6H_4	32	78	87
3–CF_3–C_6H_4	20	87	52

[a] as (*R*)–(+)–MTPA derivatives

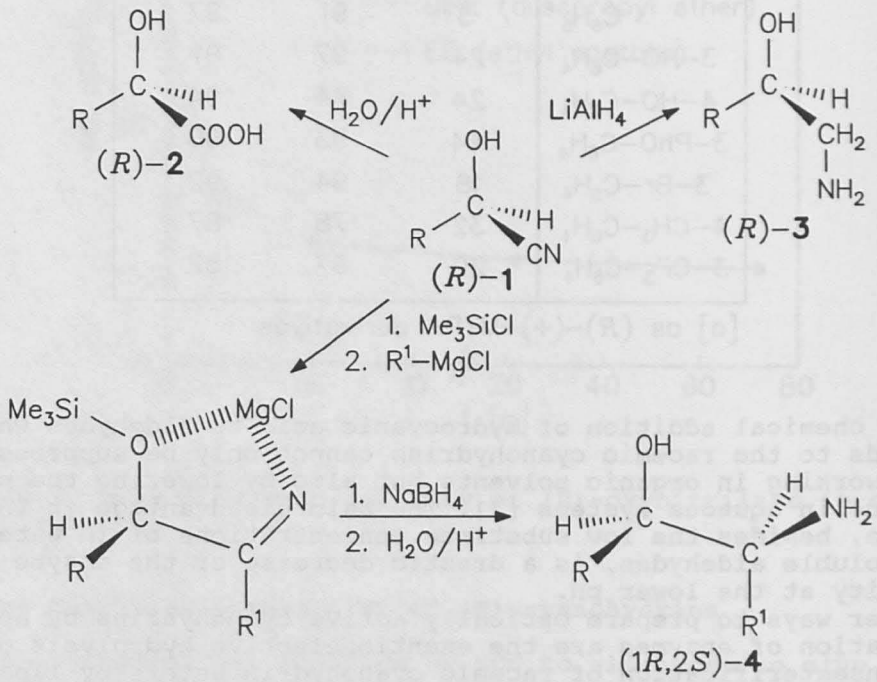
The chemical addition of hydrocyanic acid to aldehydes which leads to the racemic cyanohydrins cannot only be suppressed by working in organic solvents but also by lowering the pH value in aqueous systems [7]. The main disadvantage in this case, besides the low substrate concentrations of in water unsoluble aldehydes, is a drastic decrease of the enzyme activity at the lower pH.
Other ways to prepare optically active cyanohydrins by application of enzymes are the enantioselective hydrolysis or transesterification of racemic cyanohydrin esters by lipases and the enantioselective esterification of racemic cyano hydrins by lipases or esterases [8].
An alternative to the enzyme catalyzed enantioselective preparation of cyanohydrins is the application of cyclic dipeptides as catalysts in this reaction [9].

Stereoselective reactions of (R)-cyanohydrins

The easy access to chiral cyanohydrins of high optical purity opens the possibility for follow-up reactions to several important classes of compounds. For all follow-up reactions the stereochemical course of these reactions is decisi-

ve. We have investigated two major pathways, firstly transformations of the nitrile group of the cyanohydrins and secondly activation and substitution of their hydroxyl function. The described reactions with the (R)-cyanohydrins are completely applicable as well to the respective (S)-cyanohydrins as we could show in several examples.

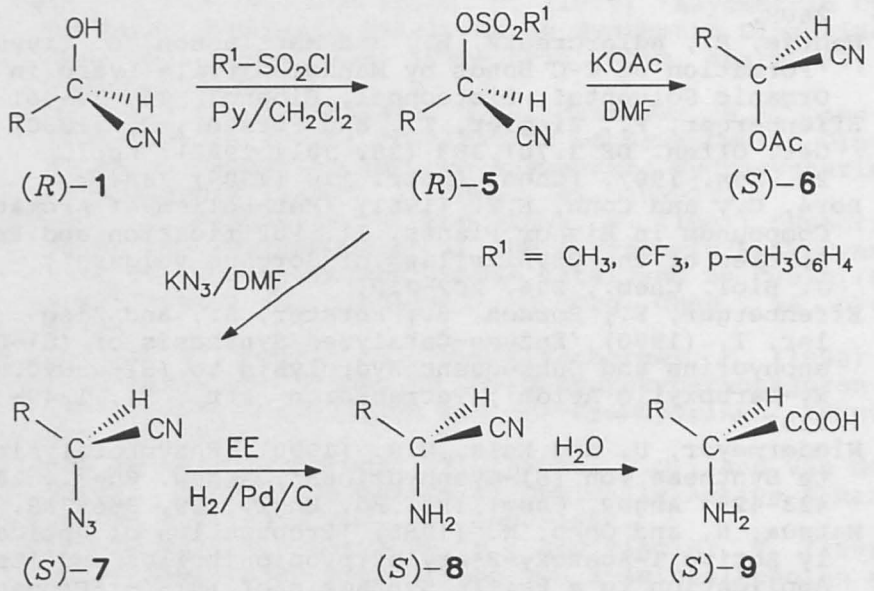
Transformations of the nitrile group of (R)-cyanohydrins



Via an acid catalyzed hydrolysis from (R)-**1**, α-hydroxy carboxylic acids (R)-**2** are obtained without any racemization in excellent yields [10]. The unprotected cyanohydrins (R)-**1** can be hydrogenated without racemization with $LiAlH_4$ to the 1,2-amino alcohols (R)-**3** (see ref. 10). The addition of Grignard reagents to the O-protected cyanohydrins and a follow-up hydrogenation with sodium borohydride leads to the (1R,2S)-amino alcohols **4**. The formations of the amino alcohols **4** occur without racemization on Carbon 1 and with very high diastereoselectivity to the erythro product with (1R, 2S) configuration [11]. Since in 1,2-amino alcohols an in-

version of the configuration on Carbon 1 is easily possible, all four stereoisomers of 1,2-amino alcohols are accessible, starting either from the (R)- or the (S)-cyanohydrin. Important compounds, like (R)-(-)-noradrenaline, (R)-(-)-adrenaline, (1R,2S)-(-)-ephedrine, can be obtained by this route (see ref.11).

Activation and substitution of the hydroxyl function of (R)-cyanohydrins



α-Sulfonyloxy nitriles (R)-**5** can be prepared from cyanohydrins (R)-**1** with sulfonyl chlorides in presence of a pyridine without racemization. The thus activated cyanohydrins (R)-**5** react with complete inversion of configuration with various nucleophiles [12]. With potassium acetate in DMF at room temperature the cyanohydrin acetates (S)-**6** are obtained in excellent yields. With potassium azide the unknown α-azido nitriles (S)-**7** are easily accessible. The azido nitriles can be hydrogenated catalytically to the respective (S)-α-amino nitriles (S)-**8** which after hydrolysis lead to the α-amino carboxylic acids (S)-**9** (see ref. 12). The sulfonyloxy nitriles (R)-**5** also react with amines as well as with sulfur and carbon nucleophiles to give the respective (S)-α-substituted nitriles.

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