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Hydroxynitrile lyase catalyzed enantioselective HCN addition to *O*-protected α-hydroxyaldehydes[1]

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Abstract

Various *O*-protected glycol- and racemic lactaldehydes **3** and **6** as well as *O*-allyl protected racemic α-hydroxyaldehydes **7** (R^1=Et, Pr, Bu) have been prepared to investigate and perform a stereoselective Kiliani–Fischer synthesis by hydroxynitrile lyase (HNL) catalyzed addition of HCN. From all protecting groups investigated the allyl moiety was most suitable. (*R*)-PaHNL from bitter almonds (*Prunus amygdalus*), yielding the (2*S*)-cyanohydrins **8**–**10**, was found to be a more stereoselective catalyst than (*S*)-MeHNL from maniok (*Manihot esculenta*). While (*R*)-PaHNL led to enantiomeric excesses ≥93%, with (*S*)-MeHNL the (2*R*)-cyanohydrins **8**–**10** were obtained with enantiomeric excesses ≤78%.

2,3-Dihydroxynitriles, readily accessible by addition of HCN to α-hydroxyaldehydes, are useful synthetic intermediates for the preparation of numerous biologically active compounds. The nitrile group can be converted by hydrogenating agents to the corresponding aldehyde which once again can be reacted with HCN to afford 2,3,4-trihydroxynitrile. This systematic chain elongation of carbohydrates has been known for more than 100 years as the Kiliani–Fischer synthesis,[2] and is often used for the preparation of polyhydroxy compounds. 1,2,3-Amino diols, also available from 2,3-dihydroxynitriles by direct hydrogenation or by Grignard addition and subsequent hydrogenation,[3] are found as components in a number of important pharmaceuticals such as β-blockers,[4] renin inhibitors[5] as well as 3-amino-2,3,6-trideoxyhexoses in anthracycline antibiotics.[6]

Nearly all biologically active and pharmacologically relevant polyhydroxy and aminohydroxy compounds have one or more stereogenic centres, and thus stereoselective syntheses of these compounds are of particular interest. By addition of HCN to aldehydes to give cyanohydrins a new stereogenic centre is generated. Thereby, as expected for reaction of a planar carbonyl function, the resultant cyanohydrins are obtained as racemates. Starting from optically active α-hydroxyaldehydes the direct HCN addition[7] as well as the reaction of bisulfite adducts of α-hydroxyaldehydes with alkali cyanides gives only very

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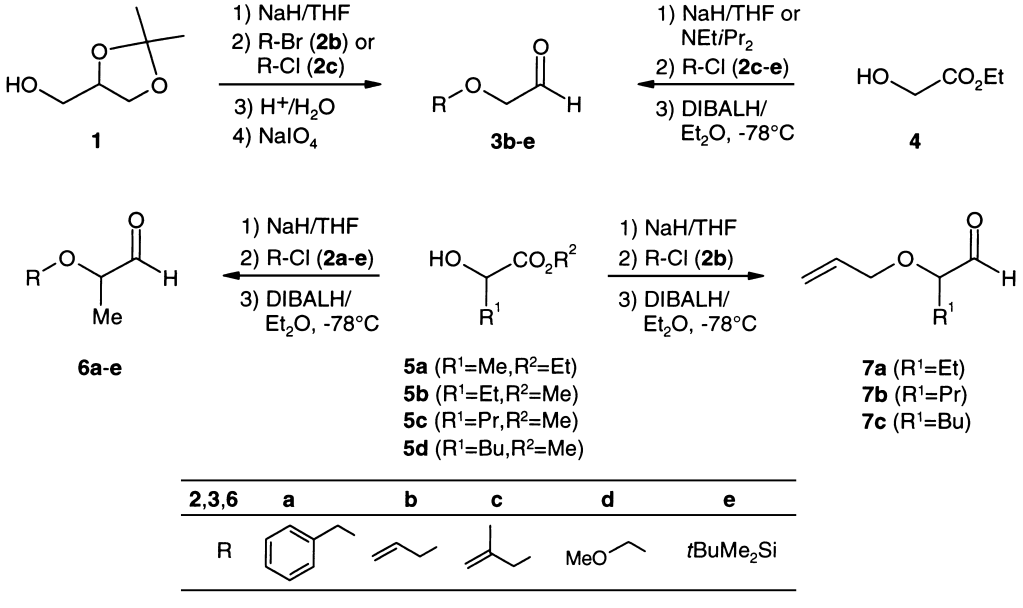
poor asymmetric induction in cyanohydrin formation.[8a,9] The cyanosilylation of optically active α-hydroxyaldehydes affords 2,3-dihydroxynitriles with higher diastereoselectivities. The use of Lewis acid catalysts[8a,10] improves the *threo*/*erythro* ratio of the trialkylsilyl cyanide addition.

In recent years many investigations have demonstrated that hydroxynitrile lyase (HNL) catalyzed additions of HCN to aldehydes proceed with high enantioselectivity.[3a] Depending on the applied HNL, (*R*)- or (*S*)-cyanohydrins are thereby easily accessible. Because of the importance of stereoselective syntheses of polyhydroxy and aminohydroxy compounds, we were interested to extend the HNL catalyzed HCN addition also to α-hydroxyaldehydes as substrates in order to perform, for example, a stereoselective Kiliani–Fischer synthesis. All investigations of this reaction have not so far been successful. Neither free nor *O*-protected α-hydroxyaldehydes were found to be substrates for the enzyme from bitter almonds ((*R*)-PaHNL).[7,8a] The (*R*)-PaHNL catalyzed HCN addition to phenoxyacetaldehyde gave only the racemic product.[11] Also under catalysis of (*S*)-HbHNL from the rubber tree (*Hevea brasiliensis*) *O*-protected hydroxyaldehydes reacted with HCN to give only racemic cyanohydrins.[12]

Following our previous experience with the HNL catalyzed HCN addition to hydroxybenzaldehydes where a strong dependence of enzymatic activity on the kind of protecting group has been found,[13] we have now investigated systematically the influence of protective groups on the HNL catalyzed addition of HCN to *O*-protected α-hydroxyaldehydes.

1. Preparation of the *O*-protected 2-hydroxyaldehydes 3, 6 and 7

The addition of HCN to α-hydroxyaldehydes bearing bulky *O*-protecting groups is not catalyzed by HNLs.[7,8a,11,12] We have therefore investigated *O*-protecting groups which are as small as possible and which can be removed easily under mild conditions (Scheme 1).



Scheme 1.

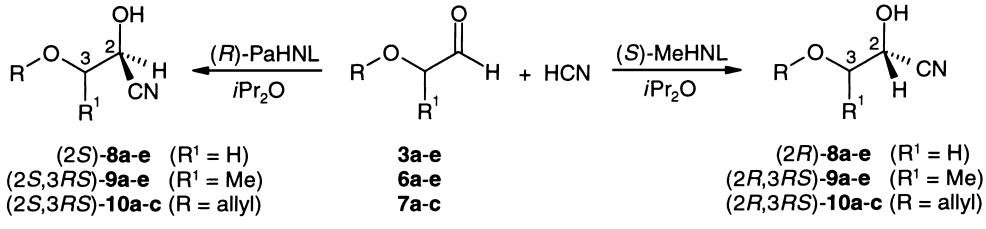
2-Benzyloxyacetaldehyde **3a** was prepared analogous to a literature method.[14] The protected glycolaldehydes 2-allyloxy- and 2-(β-methallyloxy)acetaldehyde **3b** and **3c** could be synthesized starting from 2,3-*O*-isopropylideneglycerol **1** on the basis of a known procedure[15] or alternatively from 2-

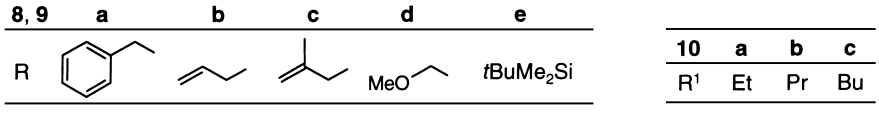
methoxymethylenoxy- and *tert*-butyldimethylsilyloxyacetaldehyde **3d** and **3e** by *O*-alkylation of ethyl hydroxyacetate **4** with alkyl halides **2** and subsequent reduction with DIBALH[16] as outlined in Scheme 1. According to this methodology the corresponding protected lactaldehydes **6a–d** were prepared from L-ethyl lactate L-**5a**[17] which, as desired for some of our investigations, is racemized by NaH during the reaction (Scheme 1). With an amine instead of NaH as base, however, as described for **6e**, racemic lactate **5a** has to be used as starting compound.[16c,d]

Analogously the 2-allyloxyaldehydes **7a–c** (with R^1=Et, Pr, and Bu) were obtained from the corresponding methyl 2-hydroxycarboxylates **5b–d** (Scheme 1) which were prepared from the respective racemic cyanohydrins via a Pinner reaction.

2. HNL catalyzed reaction of *O*-protected racemic hydroxyaldehydes 3, 6 and 7 with HCN

The *O*-protected glycol- and lactaldehydes **3** and **6** were reacted with HCN under catalysis of (*R*)-PaHNL from *Prunus amygdalus* [EC 4.1.2.10] and (*S*)-MeHNL from *Manihot esculenta* [EC 4.1.2.37] to give the cyanohydrins (2*S*)-, (2*R*)-**8** and (2*S*,3*RS*)-, (2*R*,3*RS*)-**9**, respectively (Scheme 2, Tables 1 and 2). In order to investigate the diastereoselectivity of the HNL catalyzed HCN addition, *O*-protected racemic lactaldehydes **6** as well as **7**, bearing the ethyl, propyl or butyl groups, were applied.





Scheme 2.

Table 1 shows for (*R*)-PaHNL a clear dependence of the enantiomeric excesses on the protecting group. Aldehydes **3b** and **3d**, with the small allyl- and methoxymethylene protecting groups, react with

Table 1
Preparation of (2*S*)- and (2*R*)-cyanohydrins **8** by addition of HCN to the *O*-protected glycolaldehydes **3** catalyzed by (*R*)-PaHNL and (*S*)-MeHNL, respectively

Aldehydes	(2*S*)-Cyanohydrins **8**				(2*R*)-Cyanohydrins **8**			
3	**8**	R.-time (h)	Yield (%)	*ee* (%)[a]	**8**	R.-time (h)	Yield (%)	*ee* (%)[a]
3a	**8a**	24	84	19	**8a**	24	89	17
3b	**8b**	24	66	96[b]	**8b**	9.5	65	38
3c	**8c**	5.5	36	78	**8c**	5.5	40	72
		30	78	75		30	73	68
3d	**8d**	8.0	46	96	**8d**	8.0	57	6
		24	quant.	91[c]		24	quant.	4
3e	**8e**	25	<3	–	**8e**	25	<3	–

[a] *ee*-Values determined after acetylation by gas chromatography on a β-cyclodextrin phase. [b] $[\alpha]_D^{20}$ = +20.2 (*c* 3.3, $CHCl_3$).

[c] $[\alpha]_D^{20}$ = +11.8 (*c* 1.23, $CHCl_3$).

Table 2
Preparation of (2*S*,3*RS*)- and (2*R*,3*RS*)-cyanohydrins **9** by addition of HCN to the protected lactaldehydes **6** catalyzed by (*R*)-PaHNL and (*S*)-MeHNL, respectively

Aldehydes	(2*S*,3*RS*)-Cyanohydrins **9**				(2*R*,3*RS*)-Cyanohydrins **9**			
6	**9**	R.-time (h)	Yield (%)	*ee* (%)[a]	**9**	R.-time (h)	Yield (%)	*ee* (%)[a]
6a	**9a**	24	96	83	**9a**	3	62	58
6b	**9b**	23	95	94[b]	**9b**	6	84	78
6c	**9c**	24	76	84	**9c**	24	78	43
6d	**9d**	6	quant.	59	**9d**	6	quant.	15
6e	**9e**	24	–	–	**9e**	24	–	–

[a] *ee*-Values refer to the new stereogenic centre generated at C-2 and are determined after acetylation by gas chromatography on a β-cyclodextrin phase. [b] $[\alpha]_D^{20} = +17.1$ (*c* 2.03, $CHCl_3$).

high stereoselectivity to yield (2*S*)-**8b** and (2*S*)-**8d** with 96% *ee*. Changing to the methallyl and the benzyl protecting group the enantiomeric excess decreases significantly from 78% (**8c**) to 19% (**8a**). On the contrary, using (*S*)-MeHNL as a catalyst, the methallyl-protected (2*R*)-**8c** was obtained with the best enantiomeric excess (72%) but in only 40% yield, whereas the allyl derivative **8b** results with only 38% *ee* (65% yield).

As can be seen from Table 1, cyanohydrin formation using (*R*)-PaHNL as catalyst is more enantioselective than (*S*)-MeHNL catalysis. The dependence on the *O*-protecting group is less pronounced with (*S*)-MeHNL as a catalyst. Table 1 reveals that neither the (*R*)- nor the (*S*)-HNL catalyzes the reaction with the silylated aldehyde **3e**. An inhibition of both enzymes by **3e** itself can thereby be excluded, since benzaldehyde, which is an excellent substrate for both enzymes, was converted to the respective (*R*)- and (*S*)-cyanohydrin after addition to the reaction mixture of **3e**.

The HNL catalyzed HCN addition to racemic lactaldehydes (*RS*)-**6** is of interest not only with respect to the enantioselectivity of cyanohydrin formation but also with respect to a kinetic resolution of the racemate. Since, as shown later in Section 3, kinetic resolution does not occur. This problem, however, will not be discussed further here.

Table 2 shows that the allyl protected lactaldehyde **6b** was converted by both enzymes with the highest enantiomeric excess to the corresponding cyanohydrins (2*S*,3*RS*)- and (2*R*,3*RS*)-**8b**, respectively. Also in the case of lactaldehydes, (*R*)-PaHNL exhibits a higher stereoselectivity than (*S*)-MeHNL. Again, both enzymes do not accept the silyl protected aldehyde **6e** as a substrate.

Based on these results, the (*R*)-PaHNL and (*S*)-MeHNL catalyzed HCN addition to the *O*-allyl protected 2-hydroxyaldehydes **7a**–**c**, with R^1=Et, Pr, and Bu, has been investigated. The results are summarized in Table 3.

Table 3 shows stereoselective cyanohydrin formation with (*R*)-PaHNL as a catalyst. Obviously owing to steric demand of the substrate with increasing chain length of R, the reaction is markedly decelerated going from **7b** to **7c**. This tendency was also observed for reactions with (*S*)-MeHNL which again is less stereoselective than (*R*)-PaHNL.

3. PaHNL catalyzed addition of HCN to the pure enantiomers (*R*)-6b and (*S*)-6b, respectively

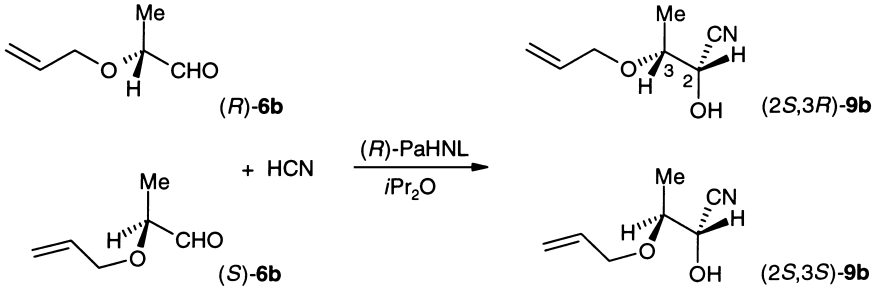
The diastereoselectivity of cyanohydrin formation was investigated for the (*R*)-PaHNL catalyzed addition of HCN to enantiomerically pure (*R*)- and (*S*)-2-allyloxypropionaldehyde (*R*)- and (*S*)-**6b**,

Table 3
Preparation of (2*S*,3*RS*)- and (2*R*,3*RS*)-cyanohydrins **10** by addition of HCN to allyloxy protected aldehydes **7** catalyzed by (*R*)-PaHNL and (*S*)-MeHNL, respectively

Aldehydes	(2*S*,3*RS*)-Cyanohydrins **10**					(2*R*,3*RS*)-Cyanohydrins **10**			
7	**10**	R.-time (h)	Yield (%)	*ee* (%)[a]	$[\alpha]_D^{20}$ (*c*, $CHCl_3$)	**10**	R.-time (h)	Yield (%)	*ee* (%)[a]
7a	**10a**	46	quant.	>99	+6.8 (0.81)	**10a**	46	quant.	37
7b	**10b**	48	98	96	+4.8 (0.67)	**10b**	48	78	55
7c	**10c**	72	91	93	+10.7 (3.5)	**10c**	72	29	23

[a] *ee*-Values refer to the new stereogenic centre generated at C-2 and are determined after acetylation by gc on a β-cyclodextrin phase.

respectively (Scheme 3), prepared from commercial D-isobutyl and L-ethyl lactate according to the literature.[16a]



Scheme 3.

The course of the HCN addition to both enantiomers was followed by gas chromatography (Fig. 1).

The conversion of the optically active aldehydes (*R*)- and (*S*)-**6b** proceeds with nearly identical reaction rates as can be seen from Fig. 1. After ca. 23 h, the amount of resultant (2*S*,3*S*)-**9b** was ca. 90% compared with ca. 80% of (2*S*,3*R*)-**9b**. Gas chromatographic analysis gave the same product ratio for the reaction of the racemic aldehyde **6b**. Thus, as shown for **6b**, both HNLs investigated are not able to

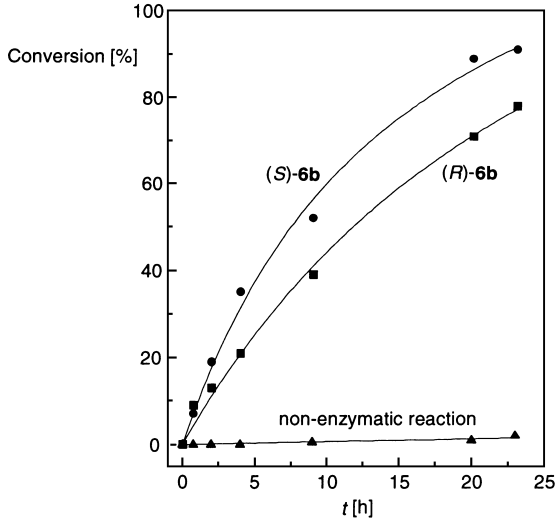


Figure 1. (*R*)-PaHNL catalyzed addition of HCN to (*R*)-**6b** (■) and (*S*)-**6b** (●), respectively

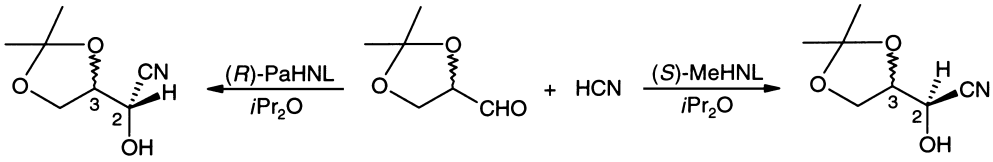
convert selectively only one enantiomer in a racemic mixture of *O*-protected α-hydroxyaldehydes to the corresponding cyanohydrin.

Summarizing the HNL catalyzed additions of HCN to *O*-protected α-hydroxyaldehydes, the allyl moiety was found to be the most suitable *O*-protective group. Using (*R*)-PaHNL as a catalyst high enantiomeric excesses (≥93%) could be achieved whereas a maximum enantiomeric excess of 78% was reached with (*S*)-MeHNL as catalyst. These results show that it is possible to prepare stereoselectively dihydroxynitriles in a Kiliani–Fischer type reaction. In the conversion of the pure enantiomers of allyloxypropionaldehyde (*R*)-**6b** and (*S*)-**6b** it could be demonstrated, for example, that both enantiomers react with HCN under (*R*)-PaHNL catalysis with comparable reaction rates. Thus, a kinetic resolution of racemic hydroxyaldehydes does not occur.

The configuration at C-2 in cyanohydrins **8**–**10** was confirmed by follow-up reactions of **6a** to a known amino deoxy sugar.[18]

4. HNL catalyzed HCN addition to *O*-protected (*R*)- and (*S*)-2,3-dihydroxyaldehyde 11

In order to perform a stereoselective Kiliani–Fischer synthesis we have investigated the enzyme catalyzed addition of HCN to isopropylidene-protected (*R*)- and (*S*)-glyceraldehyde **11** (Scheme 4, Table 4). Aldehyde (*R*)-**11** is easily available from D-mannitol,[19] and (*S*)-**11** can be prepared starting from L-ascorbic acid by a three-step procedure.[20]



(2*S*,3*R*)-**12**
(2*S*,3*S*)-**12**

(*R*)-**11**
(*S*)-**11**

(2*R*,3*R*)-**12**
(2*R*,3*S*)-**12**

Scheme 4.

The non-enzymatic addition of HCN to both (*R*)- and (*S*)-**11** shows a stereopreference for the *threo* diastereomer (2*R*,3*R*)- and (2*S*,3*S*)-**12**, respectively, with 19% *de* and 16% *de* (Table 4). The (*R*)-PaHNL catalyzed reaction of (*R*)-**11** with HCN yields the trihydroxynitrile (2*S*,3*R*)-**12** with 60% *de*, whereas the enantiomer (*S*)-**11** reacts to give (2*S*,3*S*)-**12** with 82% *de*. The higher diastereoselectivity of the (*R*)-PaHNL catalyzed reaction of (*S*)-**11** can be explained by also taking into account the chemical addition.

Table 4
Formation of (2*S*,3*R*)/(2*S*,3*S*)- and (2*R*,3*R*)/(2*R*,3*S*)-cyanohydrins **12** by HCN addition to protected glyceraldehyde (*R*)- and (*S*)-**11** catalyzed by (*R*)-PaHNL and (*S*)-MeHNL, respectively

Aldehyde **11**	Hydroxynitrile lyase	(U/mmol **11**)	R.-time (h)	Trihydroxynitrile **12** **12**	Conv. (%)	*de* (%)
(*R*)	PaHNL	333	26	(2*S*,3*R*)	85	60
(*R*)	MeHNL	200	26	(2*R*,3*R*)	quant.	22
(*R*)	–[a]	–	26	(2*R*,3*R*)	quant.	19
(*S*)	PaHNL	255	20.25	(2*S*,3*S*)	99	82
(*S*)	MeHNL	135	20.25	(2*R*,3*S*)	99	7
(*S*)	–[a]	–	20.25	(2*S*,3*S*)	85	16

[a] Non-enzymatic chemical HCN addition for comparison.

In the case of (*S*)-**11** the stereo-induction is in the same direction as the (*R*)-PaHNL catalyzed reaction, whereas in the case of (*R*)-**11** it is inverse.

With (*S*)-MeHNL as catalyst the reaction of (*R*)-**11** with HCN gives (2*R*,3*R*)-**12** with 22% *de*, which is in the range of the non-enzymatic chemical reaction (19% *ee*), whereas with (*S*)-**11** only a very low diastereoselectivity (7% *de*) for (2*R*,3*S*)-**12** is observed. From these results a low activity of the enzyme for the substrates (*R*)- and (*S*)-**11** can be deduced. Therefore the diastereoselectivity of the non-enzymatic reaction compensates the enzyme catalyzed reaction.

The configuration of the stereogenic centre generated at C-2 could be established by comparison of ^{13}C NMR spectra of commercially available pure D-erythronic acid γ-lactone and that derived from (2*S*,3*R*)-**12** by treatment with conc. HCl.

5. Experimental

5.1. Materials and methods

Benzyloxyacetaldehyde **3a** was prepared according to a literature method.[14] D-Isobutyl lactate was purchased from Degussa AG, L-ethyl lactate from Fluka, and D-erythronic acid γ-lactone from Aldrich. Melting points were determined on a Büchi SMP-20 and are uncorrected. ^1H NMR spectra were recorded on a Bruker AC 250 F (250 MHz) and ARX 500 (500 MHz) with TMS as internal standard. Optical rotations were determined in a Perkin–Elmer polarimeter 241 LC. GC for determination of enantiomeric and diastereomeric excess: (a) Hewlett–Packard 5890 Series II with FID, 0.45 bar hydrogen, column 30 m×0.32 mm, phase OV 1701; (b) Hewlett–Packard 6890 Series with FID, 0.9 bar hydrogen, column 30 m×0.32 mm, phase Chiraldex B-TA (ICT); (c) Carlo Erba MRGC 5300 Mega Series with FID, 0.4 bar hydrogen, column 20 m, phase Bondex-unβ-5.5-Et-105.

5.2. Preparation of allyl- and methallyloxyacetaldehydes *3b*,*c* from glycerol *1*: general procedure

(a) According to known methods[15] a solution of **1** (0.11–0.2 mol) in THF (100–200 mL) was added dropwise to a suspension of NaH (0.13–0.24 mol) in THF (250–500 mL), and the reaction mixture was heated to reflux for 30 min. After cooling to room temperature, ca. 1.2 equivalents of **2b** or **2c** were added, and the reaction mixture was refluxed for a further 12 h. The solvent was removed, the residue taken up in diethyl ether, and precipitated sodium halide filtered off. The filtrate was dried ($MgSO_4$) and concentrated. The residue was fractionally distilled to give 1-allyloxy- and 1-(β-methallyloxy)-2,3-isopropylideneglycerol in 77% and 78% yield, respectively.

1-(β-Methallyloxy)-2,3-isopropylideneglycerol: bp 83°C/10 torr; ^1H NMR ($CDCl_3$): δ=1.37, 1.43 (each s, 3H, $C(CH_3)_2$), 1.73 (s, 3H, CH_3), 3.39–4.33 (m, 7H, 3 CH_2, CH), 4.90–4.96 (m, 2H, $=CH_2$).

(b) For removal of the isopropylidene protecting group a solution of the respective acetal (see above) (54–81 mmol) in 2N HCl/THF (10–30 mL/100–120 mL) was stirred at room temperature for 20–28 h (TLC control). The reaction mixture was then neutralized with solid $NaHCO_3$, and THF was removed. Dichloromethane was added followed by an equimolar solution (based on acetal) of $NaIO_4$ in water (100–130 mL) under ice cooling. After being stirred for 12 h at room temperature, the organic phase was separated and the aqueous layer extracted three times with dichloromethane (70 mL each). The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was distilled in vacuo to give **3b**[14b] and **3c** in 60 and 58% yield, respectively.

5.3. Preparation of protected hydroxyacet- and propionaldehydes **3c**,**d** and **6a**–**d** from **4** and L-**5a**, respectively: general procedure

(a) According to Drewes et al.[17] a solution of **4** or L-**5a** in THF was slowly added dropwise to a suspension of NaH in THF, and the reaction mixture was heated to 60°C for 30 min. Then a solution of **2** in THF was added dropwise. After being heated to reflux for 12 h, THF was removed and the residue taken up in diethyl ether. Sodium halide was filtered off, and the filtrate was dried ($MgSO_4$), concentrated and distilled in vacuo.

Ethyl methallyloxyacetate: bp 75°C/10 torr; ^{1}H NMR ($CDCl_3$): δ=1.29 (t, *J*=7.1 Hz, 3H, CH_2CH_3), 1.76 (s, 3H, CH_3), 4.00, 4.05 (each s, 2H, CH_2), 4.23 (q, 2H, CH_2CH_3), 4.94–4.99 (m, 2H, $=CH_2$).

Ethyl methoxymethylenoxyacetate: bp 66°C/13 torr; ^{1}H NMR ($CDCl_3$): δ=1.30 (t, *J*=7.2 Hz, 3H, CH_2CH_3), 3.41 (s, 3H, CH_3O), 4.16 (s, 2H, CH_2), 4.24 (q, 2H, CH_2CH_3), 4.72 (s, 2H, OCH_2O).

Ethyl 2-methallyloxypropionate: ^{1}H NMR ($CDCl_3$): δ=1.29 (t, *J*=7.1 Hz, 3H, CH_2CH_3), 1.42 (d, *J*=6.8 Hz, 3H, $CHCH_3$), 1.76 (s, 3H, CH_3), 3.81–4.08 (m, 3H, CH, CH_2), 4.21 (q, 2H, CH_2CH_3), 4.91–4.98 (m, 2H, $=CH_2$).

(b) Analogous to known procedures,[16a,c] to a solution of the respective protected ester in diethyl ether or *n*-hexane:THF (95:5) at −78°C a 1 M solution of DIBALH in *n*-hexane (ca. 1.1–1.4 equivalents) was slowly added dropwise, and the reaction mixture was stirred for 4–6.5 h at −78°C. After hydrolysis with water, the reaction mixture was allowed to warm to room temperature. Aluminium hydroxide was filtered off through a glass frit and washed with diethyl ether. The combined filtrates were dried ($MgSO_4$), and concentrated. The residue was distilled in vacuo to yield aldehydes **3c**,**d** and **6a**–**d**.[16a,d,17]

5.4. Preparation of silylated hydroxyaldehydes **3e** and **6e** from **4** and **5a**: general procedure

To a solution of **4** or **5a** and diisopropylethylamine in dichloromethane at 0°C **2e** was added dropwise. The reaction mixture was allowed to warm to room temperature (12 h) and washed with 1N HCl, water, sat. $NaHCO_3$ solution and water (50 mL each). The organic phase was dried ($MgSO_4$), and concentrated. The residue was distilled in vacuo. The reduction to aldehyde **3e**[21] and **6e**,[16c] respectively, was performed as described above under Section 5.3.b.

5.5. Preparation of allyl protected hydroxyaldehydes **7a**–**c**

(a) A solution of the respective racemic cyanohydrin in methanolic HCl was heated in a sealed tube to 90°C for 8 h. After being cooled to room temperature, the reaction mixture was hydrolyzed with water (20 vol%) and extracted with diethyl ether (50 mL each). The combined extracts were washed with sat. $NaHCO_3$ solution until neutral, dried ($MgSO_4$), and concentrated. The residue was distilled in vacuo to give esters **5b**–**d**.

(b) The allyl protecting group was introduced in **5b**–**d** as described above in Section 5.3.a.

Methyl 2-allyloxybutyrate: bp 63°C/10 torr; ^{1}H NMR ($CDCl_3$): δ=0.97 (t, *J*=7.4 Hz, 3H, CH_3), 1.72–1.85 (m, 2H, C^3H_2), 3.75 (s, 3H, OCH_3), 3.80–4.20 (m, 3H, CH_2, CH), 5.17–5.33 (m, 2H, $=CH_2$), 5.84–5.99 (m, 1H, =CH).

Methyl 2-allyloxypentanoate: bp 75°C/10 torr; ^{1}H NMR ($CDCl_3$): δ=0.93 (t, *J*=7.3 Hz, 3H, CH_3), 1.36–1.79 (m, 4H, $(CH_2)_2$), 3.75 (s, 3H, OCH_3), 3.83–3.94 (m, 2H, CH_2), 4.11–4.19 (m, 1H, CH), 5.17–5.33 (m, 2H, $=CH_2$), 5.83–5.99 (m, 1H, =CH).

Table 5
Physical and ^{1}H NMR data of protected hydroxyaldehydes **3**, **6** and **7**[a]

Compd[b]	bp (°C/torr)	^{1}H NMR (250 MHz, $CDCl_3$, δ)
3c	51/15	1.77 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 4.06 (d, J = 0.6 Hz, 2H, CH_2CO), 4.96-5.00 (m, 2H, $=CH_2$), 9.75 (t, 1H, CHO)
3d	31/10	3.42 (s, 3H, CH_3O), 4.20 (d, J = 0.7 Hz, 2H, CH_2), 4.72 (s, 2H, OCH_2O), 9.75 (t, 1H, CHO)
6c	63/30	1.31 (d, J = 7.0 Hz, 3H, CH_3), 1.77 (s, 3H, CH_3), 3.83 (dq, J = 1.8 Hz, 1H, CH), 3.96 (d, J = 12.4 Hz, 1H, CH_aH_b), 4.04 (d, 1H, CH_aH_b), 4.94-5.00 (m, 2H, $=CH_2$), 9.68 (d, 1H, CHO)
7a	64/33	0.99 (t, J = 7.5 Hz, 3H, CH_3), 1.65-1.80 (m, 2H, C^3H_2), 3.63-3.68 (m, 1H, CH), 4.00-4.18 (m, 2H, CH_2), 5.21-5.36 (m, 2H, $=CH_2$), 5.86-5.99 (m, 1H, =CH), 9.65 (d, J = 2.1 Hz, 1H, CHO)
7b	81/35	0.94 (t, J = 7.2 Hz, 3H, CH_3), 1.37-1.69 (m, 4H, $(CH_2)_2$), 3.72 (dt, J_1 = 2.2, J_2 = 6.4 Hz, 1H, CH), 3.97-4.19 (m, 2H, CH_2), 5.20-5.35 (m, 2H, $=CH_2$), 5.84-6.00 (m, 1H, =CH), 9.66 (d, 1H, CHO)
7c[c]	92/30	0.91 (t, J = 7.1 Hz, 3H, CH_3), 1.32-1.41 (m, 4H, $(CH_2)_2$), 1.63-1.68 (m, 2H, C^3H_2), 3.71 (dt, J_1 = 2.2, J_2 = 6.3 Hz, 1H, CH), 3.98-4.20 (m, 2H, CH_2), 5.21-5.34 (m, 2H, $=CH_2$), 5.87-5.97 (m, 1H, =CH), 9.65 (d, 1H, CHO)

[a] The data of the other hydroxyaldehydes **3** and **6** correspond with those in the literature.[14b,16,22] [b] All compounds gave correct elemental analyses or mass spectra. [c] 300 MHz spectrum.

Methyl 2-allyloxyhexanoate: bp 92°C/10 torr; ^{1}H NMR ($CDCl_3$): δ=0.90 (t, J=5.8 Hz, 3H, CH_3), 1.28–1.46 (m, 4H, $(CH_2)_2$), 1.64–1.79 (m, 2H, C^3H_2), 3.75 (s, 3H, OCH_3), 3.83–4.19 (m, 3H, CH_2, CH), 5.17–5.33 (m, 2H, $=CH_2$), 5.83–5.99 (m, 1H, =CH).

(c) Aldehydes **7** were prepared from allyl protected esters (Section 5.5.b) as described above in Section 5.3.b.

The NMR data and elemental analyses for compounds **3**, **6**–**10** are given in Tables 5 and 6.

5.6. *Enzyme catalyzed preparation of optically active cyanohydrins **8**–**10***

Compounds (2*S*)-**8** and (2*S*,3*RS*)-**9**, **10** as well as (2*R*)-**8** and (2*R*,3*RS*)-**9**, **10** were prepared by (*R*)-PaHNL [EC 4.1.2.10] and (*S*)-MeHNL [EC 4.1.2.37] catalyzed addition of HCN to aldehydes **3**, **6** and **7** analogous to literature procedures.[23]

5.7. *Determination of enantiomeric excesses*

A solution of cyanohydrin (10 μL), pyridine (10 μL) and acetic anhydride (50 μL) in 0.5 mL dichloromethane was heated to 60°C for 5 h. The reaction mixture was filtered through a silica gel column (3×0.5 cm) with ca. 4 mL dichloromethane. Enantiomeric and diastereomeric excesses were determined by gas chromatography directly from the filtrate. The acetylated derivatives were also used for elemental analyses.

Table 6
NMR data of protected cyanohydrins **8**–**10** and elemental analysis of acetylated cyanohydrins ac **8**–**10**

	^{1}H NMR (250 MHz, $CDCl_3$, δ)
8a	3.64-3.74 (m, 3H, CH_2, OH), 4.55 (t, J = 4.4 Hz, 1H, CH), 4.63 (s, 2H, CH_2Ph), 7.25-7.41 (m, 5H, H_{Ph})
8b	3.10 (bs, 1H, OH), 3.72 (d, J = 4.2 Hz, 2H, C^3H_2), 4.11-4.15 (m, 2H, CH_2), 4.59 (t, 1H, CH), 5.25-5.38 (m, 2H, =CH_2), 5.83-5.99 (m, 1H, =CH)
8c	1.77 (s, 3H, CH_3), 3.13 (bs, 1H, OH), 3.68 (d, J = 4.2 Hz, 2H, C^3H_2), 4.03 (s, 2H, CH_2), 4.59 (t, 1H, CH), 4.90-5.00 (m, 2H, =CH_2)
8d	3.50 (s, 3H, CH_3O), 3.77 (dd, J_1 = 3.2, J_2 = 11.7 Hz, 1H, CH_aH_b), 3.98 (dd, J = 3.9 Hz, 1H, CH_aH_b), 4.56 (t, 1H, CH), 4.69-4.77 (m, 2H, OCH_2O)
9a[a]	1.33 (d, J = 6.3 Hz, 3H, CH_3), 1.34 (d, J = 6.3 Hz, 3H, CH_3), 3.09 (bs, 1H, OH), 3.74-3.86 (m, 1H, C^3H), 4.32 (d, J = 4.4 Hz, 1H, C^2H), 4.36 (d, J = 3.9 Hz, 1H, C^2H), 4.49-4.76 (m, 2H, CH_2Ph), 7.30-7.41 (m, 5H, H_{Ph})
9b[a]	1.31 (d, J = 6.3 Hz, 3H, CH_3), 1.32 (d, J = 6.3 Hz, 3H, CH_3), 2.99 (bd, 1H, OH), 3.14 (bd, 1H, OH), 3.71-3.82 (m, 1H, C^3H), 3.97-4.40 (m, 3H, CH_2, C^2H), 5.22-5.36 (m, 2H, =CH_2), 5.84-6.00 (m, 1H, =CH)
9c[a]	1.31 (2 d, J = 6.3 Hz, 3H, C^4H_3), 1.78 (s, 3H, CH_3), 3.07 (bs, 1H, OH), 3.20 (bs, 1H, OH), 3.69-3.80 (m, 1H, C^3H), 3.91-4.13 (m, 2H, CH_2), 4.34 (d, J = 4.4 Hz, 1H, C^2H), 4.39 (d, J = 4.0 Hz, 1H, C^2H), 4.95-5.01 (m, 2H, =CH_2)
9d[a]	1.36 (d, J = 6.4 Hz, 3H, CH_3), 3.20 (bs, 1H, OH), 3.46 (s, 3H, CH_3O), 3.55 (s, 3H, CH_3O), 3.84 (dq, J_1 = 2.4, J_2 = 6.5 Hz, 1H, C^3H), 3.95 (dq, J_1 = 4.8, J_2 = 6.4 Hz, 1H, C^3H), 4.32 (d, J = 2.4 Hz, 1H, C^2H), 4.39 (d, J = 4.8 Hz, 1H, C^2H), 4.74 (s, 2H, OCH_2O), 4.76 (s, 2H, OCH_2O)
10a[a]	0.98 (2 t, J = 7.4 Hz, 3H, CH_3), 1.56-1.90 (m, 2H, C^4H_2), 3.15 (bd, 1H, OH), 3.24 (bd, 1H, OH), 3.48-3.57 (m, 1H, C^3H), 4.05-4.28 (m, 2H, CH_2), 4.40 (bs, 1H, C^2H), 4.48 (bs, 1H, C^2H), 5.21-5.37 (m, 2H, =CH_2), 5.85-6.02 (m, 1H, =CH)
10b[a]	0.96 (t, J = 7.2 Hz, 3H, CH_3), 0.97 (t, J = 7.2 Hz, 3H, CH_3), 1.30-1.83 (m, 4H, $(CH_2)_2$), 3.25 (bs, 1H, OH), 3.53-3.65 (m, 1H, C^3H), 4.05-4.26 (m, 2H, CH_2), 4.37 (d, J = 3.5 Hz, 1H, C^2H), 4.47 (d, J = 4.1 Hz, 1H, C^2H), 5.23-5.37 (m, 2H, =CH_2), 5.85-6.01 (m, 1H, =CH)
10c[a]	0.92 (t, J = 5.9 Hz, 3H, CH_3), 0.93 (t, J = 5.7 Hz, 3H, CH_3), 1.33-1.82 (m, 6H, $(CH_2)_3$), 2.96 (bs, 1H, OH), 3.53-3.63 (m, 1H, C^3H), 4.03-4.26 (m, 2H, CH_2), 4.37 (d, J = 3.2 Hz, 1H, C^2H), 4.47 (d, J = 3.9 Hz, 1H, C^2H), 5.23-5.37 (m, 2H, =CH_2), 5.86-6.01 (m, 1H, =CH)

	Mol. formula (Mol. weight)	Calculated/found C	H	N		Mol. formula (Mol. weight)	Calculated/found C	H	N
ac **8a**	$C_{12}H_{13}NO_3$ (219.2)	65.74 65.50	5.98 5.98	6.39 6.02	ac **9c**	$C_{10}H_{15}NO_3$ (197.2)	60.90 60.68	7.67 7.66	7.10 6.93
ac **8b**	$C_8H_{11}NO_3$ (169.2)	56.80 56.70	6.55 6.58	8.28 8.02	ac **9d**	$C_8H_{13}NO_4$ (187.2)	51.33 51.08	7.00 7.03	7.48 7.51
ac **8c**	$C_9H_{13}NO_3$ (183.2)	59.00 59.02	7.15 7.18	7.65 7.48	ac **10a**	$C_{10}H_{15}NO_3$ (197.2)	60.90 60.70	7.67 7.79	7.10 6.97
ac **8d**	$C_7H_{11}NO_4$ (173.2)	48.55 48.36	6.40 6.38	8.09 7.83	ac **10b**	$C_{11}H_{17}NO_3$ (211.3)	62.54 62.53	8.11 8.19	6.63 6.66
ac **9a**	$C_{13}H_{15}NO_3$ (233.3)	66.93 66.73	6.48 6.55	6.00 5.89	ac **10c**	$C_{12}H_{19}NO_3$ (225.3)	64.00 64.03	8.50 8.57	6.22 6.22
ac **9b**	$C_9H_{13}NO_3$ (183.2)	59.00 59.06	7.15 7.23	7.65 7.77					

[a] *Diastereomeric mixtures; the proton signals partially interfere and cannot be assigned separately.*

5.8. Enzyme catalyzed preparation of trihydroxynitrile **12** from protected (R)- and (S)-glyceraldehyde **11**

(a) (*R*)- and (*S*)-**11** was reacted with HCN in diisopropyl ether under (*R*)-PaHNL and (*S*)-MeHNL catalysis as described in the literature[23] to yield the respective diastereomers **12**.

(2*S*,3*R*)-**12**: $[\alpha]_D^{20}$=−15.5 (*c* 1.15, $CHCl_3$), 60% *de*; ^{1}H NMR ($CDCl_3$): δ=1.39, 1.51 (each s, 3H, CH_3), 3.37 (bs, 1H, OH), 3.99–4.21 (m, 2H, C^4H_2 interfered), 4.32–4.38 (m, 1H, C^3H interfered), 4.42 (d, *J*=5.6 Hz, 1H, C^2H).

(2*S*,3*S*)-**12**: $[\alpha]_D^{20}$=+9.43 (*c* 1.4, $CHCl_3$), 82% *de*; ^{1}H NMR ($CDCl_3$): δ=1.40, 1.51 (each s, 3H, CH_3), 3.23 (bs, 1H, OH), 4.02 (dd, *J*=5.1, 9.3 Hz, 1H, C^4H_2), 4.18 (dd, *J*=6.6 Hz, 1H, C^4H_2), 4.35 (dt, 1H, C^3H), 4.49 (d, *J*=4.5 Hz, 1H, C^2H). MS (Auto CI, 70 eV) for $C_7H_{11}NO_3$: calcd (MH^+) 158.0817; found 158.0814. MS: *m/z* (%): 158.1 (1) [MH^+], 142.1 (52) [$M-CH_3$], 101.0 (29) [dioxolane], 43.0 (100), 28.0 (25).

5.9. Preparation of D-erythronic acid γ-lactone from (2S,3R)-12

A solution of (2*S*,3*R*)-**12** (146 mg, 0.93 mmol) in conc. HCl (6 mL) was heated to 60°C for 3 h. The reaction mixture was evaporated, and the residue was extracted three times with ethyl acetate (5 mL each). The combined extracts were concentrated to give 77 mg (61%) D-erythronic acid γ-lactone.

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