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Synthesis of optically active cyanohydrins from aromatic ketones: evidence of an increased substrate range and inverted stereoselectivity for the hydroxynitrile lyase from *Linum usitatissimum*

Christopher Roberge,* Fred Fleitz, David Pollard and Paul Devine

Merck Research Laboratories, Merck & Co, Inc., Rahway, NJ 07065, USA

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Abstract—The synthesis of (*R*)- and (*S*)-cyanohydrins from a range of aromatic methyl and ethyl ketones, including the first examples from substituted variants of phenylacetone, benzylacetone and propiophenone, is described. Commercially available hydroxynitrile lyase (HNL) enzymes were used to catalyze the asymmetric addition of cyanide to the ketones, including the first successful application of the flax HNL (LuHNL) to the synthesis of any aromatic (*S*)-cyanohydrin. Both reaction yields and stereoselectivities were shown to be influenced by the carbon chain length between the ketone and phenyl functional groups, and the type of aromatic substitution present on the starting material. Substrates converted with the greatest degree of productivity and selectivity were phenylacetones with large, electron withdrawing *meta*-substituents, such as 3-Cl, 3-Br and 3-CF_3 phenylacetones, from which cyanohydrins are formed with 93–99% ee and 61–71% yield.

1. Introduction

Asymmetric cyanation of carbonyl compounds has become an increasingly important component of enantiospecific organic synthesis schemes. The cyanohydrin products of these reactions are of great industrial significance, because of the large number and wide variety of biologically active chiral compounds that can be synthesized from these 2-hydroxynitriles with tertiary or quaternary stereocentres.[1] By derivatizing either or both of the alcohol and nitrile functional groups of these compounds, useful synthetic building blocks including, but not limited to, ketones, amines, esters and nitriles can be formed without a loss of enantiomeric purity.

An increasing array of catalysts, including cyclic dipeptides, transition metal complexes and enzymes, have been identified as being applicable for the stereospecific generation of cyanohydrins from a wide variety of aldehyde starting materials.[2,3] Asymmetric conversion of ketones to tertiary cyanohydrins has however proven to be much more challenging, in large part due to the lower reactivity of ketone compounds relative to that of analogous aldehydes, and the higher steric congestion adjacent to the carbonyl group adding to the difficulty of enantiospecific cyanide addition. The past decade has seen many advances in the discovery and development of chemical catalysts applicable to the asymmetric formation of cyanohydrins from ketone starting materials.[2,4] Despite these improvements, there are still significant obstacles to the practical application of these chemical catalysts in several cases. There are often long synthetic routes, with as many as thirteen linear steps, necessary for the production of metallic bifunctional catalysts.[5] The reaction conditions frequently involve extreme reaction conditions, with temperatures as low as −80 °C.[4] Also, the catalyst loading is typically high, with as much as 20 mol % of the ligand required for the production of cyanohydrin with adequate chiral purity.[2] Finally, most applications of chemical catalysts produce the trimethylsilylated product rather than the true cyanohydrin. Protection of the alcohol functionality prevents subsequent single-step acylations, esterifications or Mitsunobu reactions that have been demonstrated in the syntheses

* Corresponding author. Tel.: +1 908 594 5059; fax: +1 908 594 5091; e-mail: christopher_roberge@merck.com

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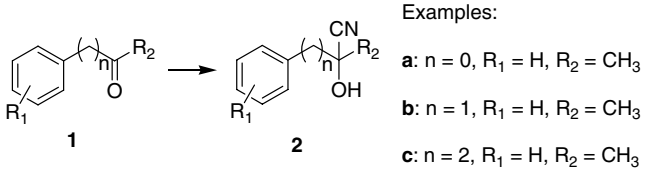
of optically active α-amino and tetronic acids from cyanohydrin building blocks.[6–8]

Alternative catalysts are the hydroxynitrile lyase (HNL) enzymes.[9–12] These biocatalysts are particularly useful in synthesizing true cyanohydrins while functioning at relatively mild conditions and exhibiting a broader substrate range and greater degree of enantioselectivity than analogous chemical catalysts. As with chemical catalysis, the large majority of published examples of HNL-catalyzed cyanohydrin formation involve the conversion of aromatic or aliphatic aldehyde compounds that share much structural similarity with the enzymes' natural substrates.[10] Additionally, there is a growing body of literature describing processes using HNLs to synthesize aliphatic ketone cyanohydrins.[13–15] However, the current number of references to the biocatalytic conversion of methyl phenyl or ethyl phenyl ketones to cyanohydrins is surprisingly small. Herein, we survey a greater spectrum of aromatic ketone substrates for HNL biocatalysis than has been discussed in the current literature, and demonstrate for the first time that HNL isolated from flax (LuHNL) is capable of converting this class of compounds into optically enriched cyanohydrins, some with ee values as high as 99%. Among these are what we believe to be the first descriptions of chiral cyanohydrin synthesis from substituted derivatives of phenylacetone, benzylacetone and propiophenone. Surprisingly, these reactions are shown to not demonstrate the exclusive (*R*)-selectivity that has been a feature of all previously known transformations catalyzed by LuHNL. The activities of two additional commercially available enzymes, PaHNL (isolated from almonds) and MeHNL (isolated from cassava), towards these aromatic ketone substrates are also discussed.

2. Results and discussion

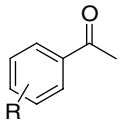
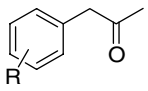
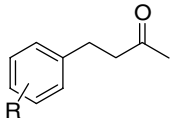
As a first step in screening a large number of aromatic ketones to identify substrates suitable for enantiospecific cyanohydrin formation with LuHNL biocatalysis, several monosubstituted derivatives of acetophenone **1a** (Scheme 1), phenylacetone **1b** and benzylacetone **1c** were screened under a set of standard reaction conditions.[16] The results of this substrate screen are summarized in Table 1.



Scheme 1. Synthesis of cyanohydrins from methyl phenyl and ethyl phenyl ketones.

The first major result of this substrate screen was that the cyanohydrins formed with the LuHNL biocatalyst are of an (*S*)-configuration. This is in contrast to all prior literature descriptions of the enzyme, involving the exclusive transformation of aliphatic aldehydes and ketones into (*R*)-cyanohydrins. Additionally, although recent discoveries in the field of chemocatalysis have led to processes of varying practicality for the production of enantiomerically enriched cyanohydrins from benzylacetone,[17,18] acetophenone[19,20] and its substituted forms,[19–21] we believe this to be the first description of the formation of chiral cyanohydrins from substituted forms of phenylacetone.

Table 1. Conversion of methyl phenyl ketones with LuHNL at 20 °C[a]

Entry	Ketone		Yield[b] (%)	ee[b] (%)	Configuration[c]
1		R = H	6	<10	Racemic
2		R = 4-F	0	—	—
3		R = 4-Br	0	—	—
4		R = 4-CH_3	0	—	—
5		R = 4-CH_3O	0	—	—
6		R = 2-Br	3	<10	Racemic
7		R = 3-F	47	59	*S*
8		R = 3-Cl	33	78	*S*
9		R = 3-Br	62[d]	93	*S*
10		R = 3-CH_3	61	91	*S*
11		R = 3-CF_3	44	95	*S*
12		R = 3-CH_3O	45	77	*S*
13		R = 4-Br	34	76	*S*
14		R = 4-CH_3O	59	66	*S*
15		R = H	15	40	*S*
16		R = 4-OH	41	36	*S*
17		R = 4-CH_3O	10	56	*S*

[a] In each reaction, 30 μL ketone substrate, 720 μL of 0.1 M citrate (pH 4.5), 100 μL LuHNL, 100 μL DIPE and 50 μL trimethylsilyl cyanide were combined and incubated for 17 h at 20 °C.

[b] Determined by normal phase chiral HPLC analysis.

[c] The absolute configuration was determined by comparison with reported data for (*S*)-cyanohydrins produced by MeHNL from acetophenone, phenylacetone and benzylacetone.[22]

[d] Confirmed with isolated yield value from 1 g reaction.

A general trend that is apparent from the data involves the effect of the distance between the phenyl and ketone functional groups of a compound on the activity of the HNL enzyme towards the substrate. The biocatalyst was shown to be most active towards derivatives of phenylacetone (entries 6–14), converting 30–65% of 8 out of 9 tested starting materials to the (*S*)-cyanohydrin with 55–95% ee in less than 1 day. Derivatives of benzylacetone (entries 15–17) were found to be poorer substrates for the enzyme, yielding conversion rates and product ee values of <40%. LuHNL exhibited the poorest activity towards acetophenone and its monosubstituted forms (entries 1–5), converting very little of these compounds to cyanohydrin product with negligible enantiomeric enhancement. As further specific examples involving identical substitution patterns, the conversion of 4-bromophenylacetone (entry 13) gave much better results than that of 4-bromoacetophenone (entry 3), which did not show appreciable reactivity. Also, conversion of 4-methoxyphenylacetone (entry 14) gave a significantly higher reaction rate and cyanohydrin ee than conversion of 4-(4-methoxyphenyl)-2-butanone (entry 17), which in turn was a better substrate than 4-methoxyacetophenone (entry 5). These results share a similar pattern with those previously published for biocatalysis with the MeHNL enzyme, in which higher cyanohydrin yields and ee values were obtained for the conversion of unsubstituted phenylacetone than that of either the smaller acetophenone or the larger benzylacetone,[22] suggesting that there may be an optimal ketone chain length for HNL biocatalysis.

Other steric effects of the substrate on the behavior of the LuHNL enzyme can be seen when considering the location and size of the substitutions to the aromatic ring. When bromine is present at the *ortho*-position of phenylacetone (entry 6), there is no significant production of cyanohydrin. Phenylacetone with bromine at the *para*-position (entry 13), though, is converted with an overnight yield of 34% to cyanohydrin with an ee of 76%. An analogous observation was made for the conversion of aldehyde substrates by both PaHNL and PmHNL (isolated from Japanese apricot). In the hydrocyanation of aldehydes catalyzed by those enzymes, the presence of substituents at the *ortho*-position always resulted in lower reaction rates and enantioselectivities than those seen with identical substituents at either the *meta*- or *para*-positions.[23] The best results for LuHNL-catalyzed hydrocyanation of brominated derivatives of phenylacetone were seen for the case of the *meta*-substitution (entry 9), which gave a yield nearly double that seen with the *para*-substitution and an excellent ee of 93%. Similarly, in comparing a pair of methoxy substituted phenylacetones, the ee of the cyanohydrin generated from the *m*-CH_3O ketone (entry 12) is higher than that generated from the *p*-CH_3O substrate (entry 14).

Within a given substitution position, the relative size of the substituent also appears to influence the stereoselectivity of the enzymatic reaction. This effect can be seen in a plot of the Taft steric parameter[24,25] versus cyanohydrin ee for all substituents tested, as is shown in Figure 1, illustrating a clear correlation between the substituent size and biocatalytic performance. One potential explanation for this phenomenon is that larger groups may result in a tighter fit

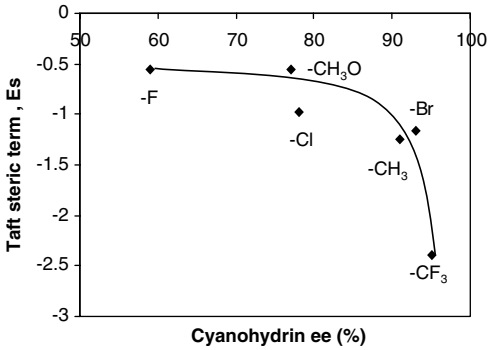


Figure 1. Correlation between Taft steric parameter of aromatic methyl ketone substitution and resulting ee of cyanohydrin formed via LuHNL biocatalysts. Reaction conditions are the same as shown in Table 1.

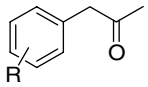
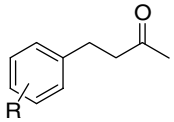
of the substrate into the active site cavity of the enzyme, enhancing the ability of the biocatalyst to discriminate between enantiomers in the process of adding cyanide to the ketone.

In addition to steric effects, the electronic nature of the substituents tested also likely plays a role in determining the enantioselectivity of the reactions. For the ketones that contain halogenated substituents, there is a monotonically increasing trend in cyanohydrin ee as the electronic withdrawing ability of the substituents is increased. This can be seen from the data listed in Table 1 for entries 7–9 and 11, describing the production of 59%, 78%, 93% and 95% ee cyanohydrins from phenylacetones with 3-F, 3-Cl, 3-Br and 3-CF_3 substitutions, respectively.

A further improvement to the biocatalytic reaction processes of Table 1 is realized by decreasing the reaction temperature from 20 to 5 °C. The result is an increase in overall chiral selectivity, as is seen in the data presented in Table 2. It is likely that this temperature reduction acts to suppress the kinetics of the background reaction of spontaneous, racemic cyanide addition more than those of the enzymatic reaction.[26] Under the new conditions, LuHNL is seen in particular to produce cyanohydrin from several of the aromatic methyl ketones (entries 3–7) at very high ee values of 93–99%.

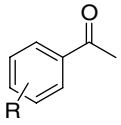
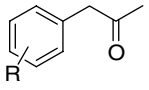
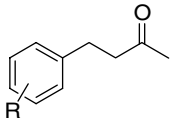
This revised set of reaction parameters was then applied to the conversion of methyl phenyl ketones by two additional commercially available HNL enzymes—MeHNL and PaHNL. Results from these conversions are given in Table 3. A comparison of the applicability of these two enzymes along with that of LuHNL shows that for most cases, higher activities towards the starting materials make MeHNL the preferred biocatalyst. Notable exceptions are the benzylacetones, the cyanohydrins of which are still generated at higher yields through MeHNL catalysis (Table 3, entries 15–17), but with much greater stereoselectivity through LuHNL catalysis (Table 2, entries 9–11). For example, LuHNL-catalyzed conversion of unsubstituted benzylacetone and 4-(4-methoxyphenyl)-2-butanone gives products with very high ee values of 89% and 99%, respectively, whereas the substrates are converted with good yields by MeHNL but to cyanohydrin that is nearly racemic.

Table 2. Conversion of methyl phenyl ketones with LuHNL at 5 °C[a]

Entry	Ketone		Yield[b] (%)	ee[b] (%)	Configuration[c]
1		R = 3-F	20	83	(*S*)
2		R = 3-Cl	24	89	(*S*)
3		R = 3-Br	37	99	(*S*)
4		R = 3-CH_3	40	97	(*S*)
5		R = 3-CF_3	19	96	(*S*)
6		R = 3-CH_3O	31	99	(*S*)
7		R = 4-Br	9	93	(*S*)
8		R = 4-CH_3O	30	84	(*S*)
9		R = H	2	89	(*S*)
10		R = 4-OH	13	75	(*S*)
11		R = 4-CH_3O	1	99	(*S*)

[a] In each reaction, 30 μL ketone substrate, 720 μL of 0.1 M citrate (pH 4.5), 100 μL LuHNL, 100 μL DIPE and 50 μL trimethylsilyl cyanide were combined and incubated for 17 h at 5 °C.
[b] Determined by normal phase chiral HPLC analysis.
[c] Absolute configuration was determined by comparison with reported data for (*S*)-cyanohydrins produced by MeHNL from acetophenone, phenylacetone and benzylacetone.[22]

Table 3. Conversion of methyl phenyl ketones with MeHNL and PaHNL at 5 °C[a]

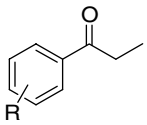
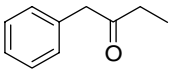
Entry	Ketone		MeHNL			PaHNL		
			Yield[b] (%)	ee[b] (%)	Configuration[c]	Yield[b] (%)	ee[b] (%)	Configuration[c]
1		R = H	9	54	(*S*)	8	97	(*R*)
2		R = 4-F	0	—	—	0	—	—
3		R = 4-Br	0	—	—	0	—	—
4		R = 4-CH_3	0	—	—	0	—	—
5		R = 4-CH_3O	0	—	—	0	—	—
6		R = 2-Br	44	97	(*S*)	1	86	(*R*)
7		R = 3-F	70	79	(*S*)	2	20	(*R*)
8		R = 3-Cl	71	93	(*S*)	1	44	(*R*)
9		R = 3-Br	61	93	(*S*)	0	—	—
10		R = 3-CH_3	65	88	(*S*)	0	—	—
11		R = 3-CF_3	67	97	(*S*)	0	—	—
12		R = 3-CH_3O	53	92	(*S*)	0	—	—
13		R = 4-Br	77	90	(*S*)	3	29	(*R*)
14		R = 4-CH_3O	62	47	(*S*)	2	<10	Racemic
15		R = H	61	7	(*S*)	2	55	(*R*)
16		R = 4-OH	54	70	(*S*)	0	—	—
17		R = 4-CH_3O	68	14	(*S*)	0	—	—

[a] In each reaction, 30 μL ketone substrate, 720 μL of 0.1 M citrate (pH 4.5), 100 μL enzyme preparation, 100 μL DIPE, and 50 μL trimethylsilyl cyanide were combined and incubated for 17 h at 5 °C.
[b] Determined by normal phase chiral HPLC analysis.
[c] Absolute configuration was determined by comparison with reported data for (*S*)-cyanohydrins produced by MeHNL from acetophenone, phenylacetone and benzylacetone.[22]

In contrast to the LuHNL and MeHNL enzymes, PaHNL exhibited very little activity towards the entire range of aromatic ketones that were examined. Only unsubstituted acetophenone, which was described previously as converting to approximately 75% ee (*R*)-cyanohydrin and 30% yield through PaHNL catalysis,[27] was transformed with >5% yield in the experiments described in this work. The significantly higher cyanohydrin ee of 97% that was observed in this case, albeit with a lower reaction yield, strongly suggests that gains in the stereoselectivity of the process are due to a decrease in the relative rate of the background hydrocyanation brought about by the choice of reaction conditions, rather than a change in the properties of the biocatalyst.

In addition to investigating the formation of chiral cyanohydrins from methyl phenyl ketones, several examples of conversion of ethyl phenyl ketones with the HNL enzymes were also tested. Data from these experiments are given in Table 4. The use of the LuHNL enzyme to produce cyanohydrins from substituted forms of propiophenone (entries 1–5) showed no measurable reaction for any of the substrate variants, a finding that is not surprising given the lack of reactivity of the enzyme towards the methyl ketone

Table 4. Conversion of ethyl phenyl ketones with LuHNL and MeHNL at 5 °C[a]

Entry	Ketone		LuHNL			MeHNL		
			Yield[b] (%)	ee[b] (%)	Configuration[c]	Yield[b] (%)	ee[b] (%)	Configuration[b]
1		R = H	0	—	—	21	90	(*S*)
2		R = 4-F	0	—	—	4	63	(*S*)
3		R = 4-Br	0	—	—	2	57	(*S*)
4		R = 4-CH_3	0	—	—	4	77	(*S*)
5		R = 4-CH_3O	0	—	—	1	88	(*S*)
6			43	89	(*S*)	59	85	(*S*)

[a] In each reaction, 30 μL ketone substrate, 720 μL of 0.1 M citrate (pH 4.5), 100 μL enzyme preparation, 100 μL DIPE, and 50 μL trimethylsilyl cyanide were combined and incubated for 17 h at 5 °C.
[b] Determined by normal phase chiral HPLC analysis.
[c] Absolute configuration was determined by comparison with reported data for (*S*)-cyanohydrins produced by MeHNL from acetophenone, phenylacetone and benzylacetone.[22]

versions of these compounds (Table 1, entries 1–5). When MeHNL was the biocatalyst used to transform the propiophenone starting materials however, conversion was observed.

3. Conclusion

The stereospecific conversion of methyl phenyl and ethyl ketones to cyanohydrins is of great synthetic utility. In the past, the insignificant activity towards aromatic ketones of PaHNL, which has to-date been the most thoroughly studied and frequently applied enzyme for chiral cyanohydrin production, may have been an impediment to the further study of this important class of biocatalytic reactions. In this work hydroxynitrile lyases, including for the first time LuHNL, have been shown to be very enantiospecific and moderately active catalysts in the hydrocyanation of a wide variety of aromatic ketones. It should be noted that the results of this initial screening work have been obtained with minimal process development, and the product ee and yield values reported represent only the minima that can be achieved. Further refinement of these processes may involve the optimization of such parameters as operating temperature and pH and the choice and concentration of organic solvent and cyanide donor used in the biphasic reaction mixtures. Another important route to explore in process improvement is the expansion of the available biocatalyst library, especially through engineering enzymes with the goal of improving activity towards ketones. As future developments bring about these activity increases, the application of HNL enzymes to organic synthesis schemes should increase significantly as well.

4. Experimental

4.1. Enzymes and chemicals

All ketone compounds and organic solvents were purchased from either Sigma–Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH). Liquid preparations of MeHNL, PaHNL and LuHNL were purchased from Julich Chiral Solutions (Julich, Germany).

4.2. General procedure for the enzymatic reaction

To 720 μL of 0.1 M aqueous citrate, pH 4.5, were added 100 μL diisopropyl ether, 100 μL liquid enzyme solution, 30 μL ketone substrate and 50 μL trimethylsilylcyanide. The reaction was then stirred at 5 °C for 17 h.

4.3. Assay of the reaction mixture

To the 1 mL reactions were added 100 μL saturated ammonium sulfate and 800 μL ethyl acetate. The mixtures were vortexed and the organic layers removed and evaporated under nitrogen. The samples were then resuspended in 600 μL isopropanol and assayed to determine both the yield and ee by chiral normal phase HPLC. A 250 mm × 4.6 mm Chiralpak AD-H column was used with an eluant of 95:5 heptane/ethanol, a flow rate of 3 mL/min, a temperature of 10 °C and a detection wavelength of 210 nm.

4.4. A 1 g scale hydrocyanation of 3-bromophenylacetone

To 18 mL of 0.1 M aqueous citrate, pH 4.5, were added 2.5 mL diisopropyl ether, 2.5 mL LuHNL solution, 750 μL 3-bromophenylacetone and 1.25 mL trimethylsilylcyanide. The reaction was then stirred at 3 °C for 24 h. The product was then extracted into solvent with the addition of 2.5 mL saturated ammonium sulfate and 25 mL ethyl acetate while stirring. The organic layer was evaporated under nitrogen, giving an oil. HPLC analysis using isolated racemic cyanohydrin as a standard, showed 770 mg of the cyanohydrin was present in the oil, resulting in a 60% yield on starting material.

4.5. 2-Hydroxy-3-(3-fluorophenyl)-2-methyl-propanenitrile (Table 1, entry 7)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.68$ (s, 3H), 2.97–3.13 (m, 2H), 7.04–7.14 (m, 4H); HPLC: t_R(ketone) = 2.5 min, t_R[(*S*)-cyanohydrin] = 4.2 min, t_R[(*R*)-cyanohydrin] = 12.2 min.

4.6. 2-Hydroxy-3-(3-chlorophenyl)-2-methyl-propanenitrile (Table 1, entry 8)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.67$ (s, 3H), 2.95–3.10 (m, 2H), 7.08–7.34 (m, 4H); HPLC: t_R(ketone) = 2.5 min, t_R[(*S*)-cyanohydrin] = 3.9 min, t_R[(*R*)-cyanohydrin] = 9.6 min.

4.7. 2-Hydroxy-3-(3-bromophenyl)-2-methyl-propanenitrile (Table 1, entry 9)

^{1}H NMR (CD_4O, 400 MHz): $\delta = 1.49$ (s, 3H), 2.95–3.00 (m, 2H), 7.14–7.50 (m, 4H); HPLC: t_R(ketone) = 2.6 min, t_R[(*S*)-cyanohydrin] = 4.0 min, t_R[(*R*)-cyanohydrin] = 10.2 min.

4.8. 2-Hydroxy-3-(3-methyl-phenyl)-2-methyl-propanenitrile (Table 1, entry 10)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.69$ (s, 3H), 2.39 (s, 3H), 2.91–3.12 (m, 2H), 7.01–7.31 (m, 4H); HPLC: t_R(ketone) = 1.9 min, t_R[(*S*)-cyanohydrin] = 3.1 min, t_R[(*R*)-cyanohydrin] = 5.7 min.

4.9. 2-Hydroxy-3-(3-trifluoromethyl-phenyl)-2-methyl-propanenitrile (Table 1, entry 11)

^{1}H NMR (CD_4O, 400 MHz): $\delta = 1.52$ (s, 3H), 2.93–3.26 (m, 2H), 7.46–7.63 (m, 4H); HPLC: t_R(ketone) = 2.0 min, t_R[(*S*)-cyanohydrin] = 2.6 min t_R[(*R*)-cyanohydrin] = 4.6 min.

4.10. 2-Hydroxy-3-(3-methoxyphenyl)-2-methyl-propanenitrile (Table 1, entry 12)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.67$ (s, 3H), 2.93–3.11 (m, 2H), 3.83 (s, 3H), 6.76–7.32 (m, 4H); HPLC: t_R(ketone) = 3.6 min, t_R[(*S*)-cyanohydrin] = 7.3 min, t_R[(*R*)-cyanohydrin] = 12.0 min.

4.11. 2-Hydroxy-3-(4-bromophenyl)-2-methyl-propanenitrile (Table 1, entry 13)

^{1}H NMR (CD_4O, 400 MHz): $\delta = 1.48$ (s, 3H), 2.93–3.01 (m, 2H), 7.23 (d, 2H, $J = 8.4$ Hz), 7.58 (d, 2H, $J = 8.4$ Hz); HPLC: t_R(ketone) = 2.9 min, t_R[(*S*)-cyanohydrin] = 4.7 min, t_R[(*R*)-cyanohydrin] = 7.1 min.

4.12. 2-Hydroxy-3-(4-methoxyphenyl)-2-methyl-propanenitrile (Table 1, entry 14)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.65$ (s, 3H), 2.89–3.08 (m, 2H), 3.82 (s, 3H), 6.88 (d, 2H, $J = 8.6$ Hz), 7.26 (d, 2H, $J = 7.8$ Hz); HPLC: t_R(ketone) = 3.6 min, t_R[(*S*)-cyanohydrin] = 6.7 min t_R[(*R*)-cyanohydrin] = 13.6 min.

4.13. 2-Hydroxy-2-methyl-4-phenylbutanenitrile (Table 1, entry 15)

^{1}H NMR (CD_4O, 400 MHz): $\delta = 1.57$ (s, 3H), 1.96–2.00 (m, 2H), 2.71–2.88 (m, 2H), 7.11–7.27 (m, 4H); HPLC: t_R(ketone) = 2.3 min, t_R[(*S*)-cyanohydrin] = 5.4 min, t_R[(*R*)-cyanohydrin] = 6.3 min.

4.14. 2-Hydroxy-2-methyl-4-(4-hydroxyphenyl)-butanenitrile (Table 1, entry 16)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.65$ (s, 3H), 2.03–2.07 (m, 2H), 2.78–2.89 (m, 2H), 6.79 (d, 2H, $J = 8.6$ Hz), 7.08 (d, 2H, $J = 8.4$ Hz); HPLC: t_R(ketone) = 1.6 min, t_R[(*S*)-cyanohydrin] = 11.0 min, t_R[(*R*)-cyanohydrin] = 27.2 min.

4.15. 2-Hydroxy-2-methyl-4-(4-methoxyphenyl)-butanenitrile (Table 1, entry 17)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.65$ (s, 3H), 2.04–2.08 (m, 2H), 2.83–2.87 (m, 2H), 3.79 (s, 3H), 6.86 (d, 2H, $J = 8.8$ Hz), 7.16 (d, 2H, $J = 8.8$ Hz); HPLC: t_R-(ketone) = 3.2 min, t_R[(*S*)-cyanohydrin] = 11.2 min, t_R[(*R*)-cyanohydrin] = 16.1 min.

4.16. 2-Hydroxy-3-phenyl-2-ethyl-propanenitrile (Table 4, entry 6)

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.20$ (s, 3H), 1.86–1.92 (m, 2H), 2.90–3.20 (m, 2H), 7.21–7.42 (m, 4H); HPLC: t_R(ketone) = 1.9 min, t_R[(*S*)-cyanohydrin] = 3.8 min, t_R[(*R*)-cyanohydrin] = 7.4 min.

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minimizing both enzyme instability that is known to be more pronounced at pH < 5 (see Albrecht, J.; Jansen, I.; Kula, M.-R. *Biotechnol. Appl. Biochem.* **1993**, *17*, 191), and the rate of non-enzymatic spontaneous cyanide addition that is increased at pH > 4 and leads to racemic product formation (see Kragl, U.; Niedermeyer, U.; Kula, M.-R.; Wandrey, C. *Ann. NY Acad. Sci.* **1990**, *613*, 617).

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