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PmHNL catalyzed synthesis of (*R*)-cyanohydrins derived from aliphatic aldehydes

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Abstract—Hydroxynitrile lyase from the Japanese apricot (*Prunus mume*) catalyzes the formation of several aliphatic cyanohydrins in an asymmetric fashion. By employing a biphasic reaction system, aliphatic aldehydes with various structural features can be converted to the corresponding (*R*)-cyanohydrins with good overall yield and enantiomeric excess.

1. Introduction

Enantiomerically pure cyanohydrins have attracted the attention of organic chemists, as well as enzymologists, due to their immense potential as chiral building blocks and interesting biological properties.[1] Enantiopure cyanohydrins serve as immediate intermediates for several industrially useful chemicals,[2] and the use of chiral cyanohydrins as building blocks for the production of important chemicals is likely to continue growing, as it avoids problems associated with the optical resolution or asymmetric synthesis of certain products. One of the most promising and interesting ways to produce enantiomerically pure cyanohydrins is the hydroxynitrile lyase (HNL) catalyzed addition of cyanide to the respective carbonyl compounds.[3] Hydroxynitrile lyase is a class of enzymes that catalyze the addition of an appropriate cyanide source to carbonyl compounds in a facial selective way, hence yielding the respective cyanohydrins with excellent enantioselection. The chemistry and biology of HNLs have been extensively explored over the past two decades,[4] and active research to find new HNLs with interesting catalytic properties is still ongoing. In our search for new HNL species, we previously reported interesting results with a new (*R*)-HNL from Japanese apricot (*PmHNL*, *Prunus mume*).[5] Herein, continuing on from this earlier report, we discuss *PmHNL* catalyzed asymmetric synthesis of cyanohydrins from aliphatic aldehydes with different structural features.

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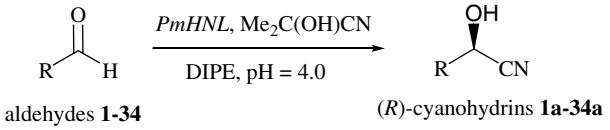
2. Results and discussion

In our previous report,[5a] we revealed that *PmHNL* accepts a broad array of carbonyl compounds as its substrate and produces the corresponding cyanohydrins with excellent enantioselection. In this earlier study, it was mainly aromatic aldehydes that were tested as possible *PmHNL* substrates with a few aliphatic aldehydes and aliphatic methyl ketones. The aliphatic aldehydes were shown to act as excellent substrates compared to the aliphatic methyl ketones. This finding prompted us to investigate a larger number of aliphatic aldehydes with different structural features as possible *PmHNL* substrates (Scheme 1). In general, a vigorously stirred biphasic reaction media was employed,[6] with acetone cyanohydrin as the cyanating source (carbonyl compounds: 5 g and 1.5 equiv of cyanating species; see Experimental for details).



aldehydes **1-34** (*R*)-cyanohydrins **1a-34a**

Scheme 1. *PmHNL* catalyzed (*R*)-cyanohydrin synthesis from aliphatic aldehydes.

2.1. Saturated aliphatic aldehydes

A series of aliphatic aldehydes were employed as the *PmHNL* substrate. Aldehydes with 3 (propanal) to 10 carbon units (decanal) were tested for *PmHNL* catalyzed cyanohydrin synthesis. By increasing chain length

from propanal to decanal, the enzyme had a steady effect on enantioselection. In agreement with our previous article,[5a] the short chain aldehydes propanal **1**, butyraldehyde **2**, pentanal **6**, and hexanal **7** were excellent *PmHNL* substrates, and all provided excellent enantioselection of the synthesized cyanohydrins with overall good chemical yield (Table 1). In contrast, with the long chain aldehydes heptanal **8** to decanal **10**, a steady decrease in enantioselectivity was observed, and decanal provided an enantioselectivity of only 12% under similar reaction conditions. By increasing the chain length, the reaction time was largely enhanced to obtain an appreciable chemical yield of the synthesized cyanohydrins. The reason for this trend remains unclear, but a similar trend was also observed with the most widely known HNL, *PaHNL* (*Prunus amygdalus*; almond).[7] When subjected to *PmHNL* catalyzed cyanohydrin synthesis, isobutyraldehyde **3**, pivalaldehyde **4**, and isovaleraldehyde **5** revealed excellent enantioselection with good chemical yield. The absolute configurations of the product cyanohydrins were confirmed as (*R*) by comparing their specific rotation values with the known compounds reported in the literature.[7,8]

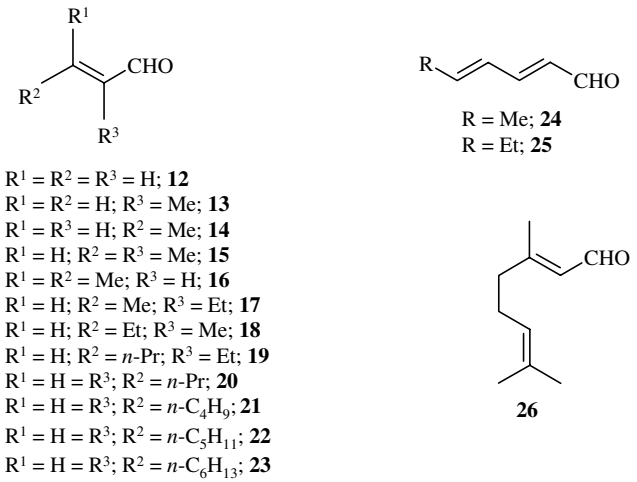
Table 1. *PmHNL* catalyzed (*R*)-cyanohydrin synthesis from aliphatic aldehydes

Substrate[a]	Time (h)	Yield (%)	ee (%)[b]
Propionaldehyde **1**[c]	11	68	94
Butyraldehyde **2**	14	58	90
Isobutyraldehyde **3**[c]	10	62	94
Pivalaldehyde **4**[c]	52	6	96
3-Methyl-1-butanal **5**[c]	13	65	95
Pentanal **6**	16	57	88
Hexanal **7**	60	22	90
Heptanal **8**	28	56	82
Octanal **9**	36	58	52
Nonanal **10**	54	42	20
Decanal **11**	72	38	12

[a] Data for compounds **1**–**4**, and **6**–**7** were taken from our previous article.[5a]
[b] Ee was determined by chiral GC analysis by derivatizing the cyanohydrins to the corresponding –TMS ethers.
[c] These cyanohydrins were not derivatized as all were clearly resolved under the GC conditions.

2.2. Unsaturated aliphatic aldehydes

A series of unsaturated aliphatic aldehydes with different structural features were also tested as *PmHNL* substrates (Scheme 2). The simplest unsaturated aldehydes, acrolein, and methacrolein **12** and **13** reacted rapidly under the reaction conditions, with an almost quantitative yield achieved at 8–10 h. However, the product cyanohydrin showed poor enantioselectivity (Table 2). The reason for this was unclear, but a plausible explanation is the high water solubility of these two compounds, the chemical cyanation of which caused poor enantioselection. It had been established that during biphasic HNL catalyzed cyanohydrin synthesis, the reaction mainly occurs in the interphase and the hydrophobicity of the substrates play an important role.[8] However, hydrophobicity alone could not be the determining factor for enantioselectivity as 2-butenal **14** provided 96% enantioselectivity, even though its hydrophobic parameters are similar to those of methacrolein **13**. Thus, hydrophobicity as well as the structural parameters of the substrate both have a pertinent effect on enantioselection. This will be discussed later.



Scheme 2. Aliphatic unsaturated aldehydes as *PmHNL* substrates.

Table 2. *PmHNL* catalyzed (*R*)-cyanohydrin synthesis from unsaturated aliphatic aldehydes

Substrate	Time (h)	Yield (%)	ee (%)[a]
Acrolein **12**	8	90	42
2-Methyl-2-propenal **13**	10	88	32
(*E*)-2-Butenal **14**	14	70	96
(*E*)-2-Methyl-2-butenal **15**	14	78	90
3-Methyl-2-butenal **16**	12	72	92
(*E*)-2-Ethyl-2-butenal **17**	48	50	92
(*E*)-2-Methyl-2-pentenal **18**	70	58	96
(*E*)-2-Ethyl-2-hexenal **19**	78	48	92
(*E*)-2-Hexenal **20**	58	62	94
(*E*)-2-Heptenal **21**[b]	76	52	72
(*E*)-2-Octenal **22**[b]	78	48	21
(*E*)-2-Nonenal **23**[b]	82	40	12
(*E*,*E*)-2,4-Hexadienal **24**[b]	56	32	96
(*E*,*E*)-2,4-Heptadienal **25**[b]	70	38	97
Geranial **26**[b]	52	48	98

[a] Ee was determined by chiral GC analysis of the cyanohydrins as they were clearly resolved under the GC conditions.
[b] These cyanohydrins were derivatized to the corresponding –TMS ethers prior to GC analysis.

3-Methyl-2-butenal and 2-methyl-2-butenal **16** and **15** were both shown to be excellent substrates of *PmHNL*, producing corresponding cyanohydrins with very good enantioselection and yield. 2-Ethyl-2-butenal, *trans*-2-methyl-2-pentenal and (*E*)-2-ethyl-2-hexenal **17**–**19** required a longer reaction time for an appreciable chemical yield (as indicated by TLC), but good enantioselection was observed with the synthesized cyanohydrins (Table 3, **17a**–**19a**). Under the reaction conditions, (*E*)-2-hexenal **20** resulted in cyanohydrin formation with excellent enantioselection; however, after 58-h reaction time, only a 62% yield of respective cyanohydrin was

Table 3. *PmHNL* catalyzed cyanohydrin synthesis from cyclic aldehydes

Substrate[a]	Time (h)	Yield (%)	ee (%)[b]
Cyclopropanecarbaldehyde **27**	10	18	Nd
Cyclobutanecarbaldehyde **28**[b]	16	78	92
Cyclopentanecarbaldehyde **29**[b]	14	70	94
Cyclohexanecarbaldehyde **30**[b]	12	72	93
Cyclohex-1-enecarbaldehyde **31**[b]	21	54	90
Cyclohex-3-enecarbaldehyde **32**[c]	16	70	96, 94
Bicyclo[2.2.1]-hept-5-ene-2-carbaldehyde[c,d] **33**	28	62	89, 81
(1*R*)-Myrtenal **34**[b]	14	82	99

[a] Data for compounds **29** and **30** was taken from our previous article.[5a]
[b] Ee was determined by chiral GC analysis; these cyanohydrins were not derivatized as all were clearly resolved under the GC conditions.
[c] The ee for **32** and **33** (enantiomeric excess at the carbonyl carbon; molar percentage excess of 2*R*,3*S* over 2*S*,3*S* and 2*R*,3*R* over 2*S*,3*R*) was determined with the help of chiral GC by derivatizing the cyanohydrins to the corresponding –TMS ethers. With the cyanohydrin–TMS ether of **32**, the composition of the four diastereomers was 49:1:48.5:1.5 and for **33** was 44.5:2.5:47.8:5.2.
[d] The starting material contained exo aldehyde (racemic) exclusively.

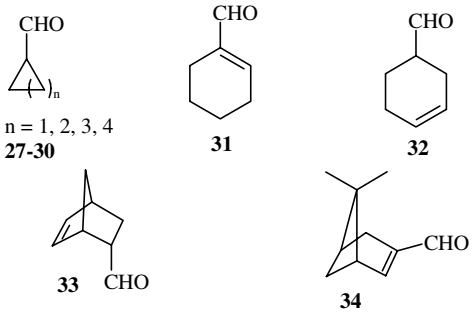
achieved. (*E*)-2-Heptenal **21** produced the corresponding cyanohydrin with moderate enantioselection (72%), whereas the higher chain aldehydes (*E*)-2-octenal and (*E*)-2-nonenal **22** and **23** both provided very poor enantioselectivity.

Increasing the chain length had a pronounced influence, lowering the ee; this was also observed in the case of the corresponding saturated aliphatic aldehydes. It was also revealed that with an increase in chain length, the aldehydes required a longer reaction time to produce an appreciable amount of products (although far below the expected level). With an increase in chain length, i.e. carbon number, the hydrophobic fragmental constant value[9] also increased. Therefore, if hydrophobicity was the only factor responsible for enantioselection, we should observe an enhanced ee value with an increase in chain length, but in fact the opposite occurred. Moreover, surprisingly, (*E*)-geranial **26**, even though it contains 10 carbon atoms, also provided excellent enantioselection, although low chemical yield of the corresponding cyanohydrin was observed. Thus, structural parameters of the substrate also seem to play an important role in governing the ee value.

The geometry (orientation) of (*E*)-geranial, even though it contains 10 carbon atoms, makes it sufficiently flexible to fit in the *PmHNL* active site. Although no structural data is currently available, as *PmHNL* is a relatively new enzyme, it seems certain that one has to consider the hydrophobic parameters as well as structural features of a given set of substrates when considering the enantioselectivity of the reaction. Substrates with an additional conjugated double bond (e.g., **24** and **25**) are also accepted by *PmHNL*, but require a sufficiently longer reaction time and result in a low chemical yield of cyanohydrins. However, with both **24** and **25**, excellent enantioselection was observed (Table 2). The absolute configurations of the product cyanohydrins were assigned to (*R*) and confirmed by comparing their specific rotation values with known compounds reported in the literature.[6–8] For compounds **17a**–**19a** and **25a**, the absolute configurations were assigned by analogy since the specific rotation values of these compounds are not reported elsewhere.

2.3. Cyclic aldehydes

We also tested several cyclic aldehydes with different structural features as *PmHNL* substrates (Scheme 3). The smallest cyclic aldehyde, cyclopropane carbaldehyde **27**, was not accepted by *PmHNL* as a substrate, and under the reaction conditions, yielded the corresponding cyanohydrin with almost no enantioselection. The chemical reactivity of cyclopropane carbaldehyde was also very low: after 10 h incubation, a yield of only 18% of respective cyanohydrin was observed. Higher aldehydes in the same series, cyclobutane carbaldehyde **28**, cyclopentane carbaldehyde **29**, and cyclohexane carbaldehyde **30**, were all excellent substrates of *PmHNL*,[5a] and all provided cyanohydrins with excellent enantioselection (Table 3) and good chemical yield. It was also evident that cyclic aldehydes are better substrates for *PmHNL* compared with their linear counterparts in terms of chemical yield as well as enantioselectivity (Tables 1 and 3).



Scheme 3. Cyclic aldehydes as *PmHNL* substrates.

Cyclohex-1-enecarbaldehyde **31**, when treated with *PmHNL* under similar reaction conditions, showed product cyanohydrins with good enantioselectivity, but low chemical yield (54%) compared with its saturated counterpart. Cyclohex-3-enecarbaldehyde **32** provided excellent enantioselection and was our first experience with a substrate with a preexisting stereogenic center (adjacent to the –CHO group). Both enantiomers of cyclohex-3-enecarbaldehyde (1*R* and 1*S*), when reacted with *PmHNL*, resulted in product cyanohydrins with

good chemical yield (70%). The enantioselectivity (enantioselection at the prochiral carbonyl group) of the reaction was measured with chiral-GC and compared with racemic samples synthesized by chemical methods. In the racemic cyanohydrin, the –TMS ether of **32** showed four peaks with characteristic baseline separation in an equimolar ratio, whereas the GC diagram of the enzymatic hydrocyanation reaction products obtained from **32** showed two major and two minor peaks (Table 3). The two major peaks [corresponding to (2*R*,3*S*) and (2*R*,3*R*)-isomers, but we are not sure, which peaks corresponded to which isomer] existed in almost equimolar amounts, indicating that the preexisting stereogenic center in the starting aldehydes is not differentiated by *PmHNL* as it accepts both enantiomers as a substrate, yielding respective cyanohydrins (by *Si*-facial addition of the CN source to both the 1*R* and 1*S* isomers of compound **32**).

In an earlier communication by Brussee et al.,[10] the (*R*)-*PaHNL* catalyzed the synthesis of cyanohydrins from substrate **32** was examined by employing aqueous EtOH as a cosolvent. However, the poor enantioselectivity of the process made it inefficient and presented the scope for us to examine our enzyme system using *PmHNL*, which subsequently provided excellent enantioselection. In another example, the *exo*-isomer of bicyclo[2.2.1]-hept-5-ene-2-carbaldehyde (racemic mixture, **33**), when reacted with *PmHNL*, resulted in good overall enantioselection (at the prochiral carbonyl group) (Table 3). It was previously reported that the two enantiomers of racemic aldehydes substituted at the α-position reacted differently with *PaHNL* catalyzed asymmetric hydrocyanation, but that the enzyme cannot distinguish between the enantiomers of racemic aldehydes devoid of any α-substitution.[11] Moreover, substitution at the β-position had absolutely no effect on the reactivity of the two enantiomers.[11] A substrate structurally related to **32** (1,2,3,4-tetrahydronaphthalene-2-carbaldehyde) was previously subjected to *PaHNL* catalyzed hydrocyanation by Riva et al.,[12] who revealed that both enantiomers were active and that the enantioselectivity was not effected by the existing chirality of the molecule. It can, therefore, be concluded that, in general, both enantiomers of a racemic aldehyde (without any α-substitution) are accepted by *PaHNL*. As our *PmHNL* system poses striking similarities in reactivity as well as enantioselectivity, *PmHNL* is expected to exhibit a similar trend, and indeed did so in the case of aldehydes **32** and **33**. Further experimental work is required to confirm the above concept and is currently under progress in our laboratory.

The last example in this series was 1(*R*)-myrtenal **34**, which has a similar bicyclic framework as norbornene aldehydes **33**, with two extra geminal –Me groups in the bridge. Under similar reaction conditions, 1(*R*)-myrtenal provided the corresponding cyanohydrins with excellent chemical and optical yield (Table 3). Thus, bicyclic substrates also seem to be accepted by the *PmHNL* active site. The absolute configurations of the product cyanohydrins of **29**, **30**, **31**, and **34** were confirmed as (*R*) by comparing their specific rotation values with known compounds reported in the literature,[1f,7,8] whereas those of all other cyanohydrins was assigned by analogy.

3. Conclusion

A careful literature survey of (*R*)-HNL catalyzed aliphatic cyanohydrins synthesis revealed that *PaHNL* is a widely explored HNL in biocatalytic asymmetric synthesis.[3] HNL from defatted apple meal and *LuHNL* (*Linum usitatissimum* hydroxynitrile lyase from flax seedlings) were also used in a few cases.[13,14] However, the reactivity and substrate specificity of HNL from apple meal and *LuHNL* were restricted to very few aliphatic aldehydes, unlike *PaHNL*, which accepts various aliphatic aldehydes, saturated, unsaturated, and cyclic, and yields respective cyanohydrins with good to excellent enantioselection and chemical yield.[3b] In our earlier report,[5a] we revealed that *PmHNL* possesses a striking similarity with *PaHNL* with regards to substrate specificity (chemical yield and enantioselectivity). Comparing the results presented here with those obtained previously with *PaHNL* catalyzed aliphatic cyanohydrin synthesis, an excellent analogy was found with respect to both chemical yield and enantioselection. It is important to point out, however that a few substrates **17**–**19**, **25**, **28**, **29**, and **33** tested here with *PmHNL* were new and have not been examined with *PaHNL* or any other HNL catalyzed cyanohydrin synthesis.

We can conclude that the compatibility of *PmHNL* with the novel substrates presented herein will provide the opportunity for further research focusing on its catalytic behavior with customized substrates. Further studies directed toward the enzymological properties of *PmHNL* will also allow the prediction of an efficient model for determining the structure–activity relationship of these kinds of enzymes.

4. Experimental

4.1. General

Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Ripen Ume fruit (*P. mume*) was obtained from a local fruit market and stored at 4 °C. All aldehydes were freshly distilled or washed with aq $NaHCO_3$ solution to minimize the amount of free acid, which is thought to inhibit HNL activity. Mandelonitrile and acetone cyanohydrins were freshly distilled prior to use. Reactions were monitored by TLC carried out on 0.25 mm thick silica gel plates (Merck) with UV light, ethanolic vanillin, and phosphomolybdic acid/heat as developing agents. Silica gel 100–200 mesh was used for column chromatography. Chemical yield (isolated yield) refers to chromatographically and spectroscopically homogeneous materials unless otherwise stated. NMR spectra were recorded on a 400 MHz spectrometer (JEOL JNM-CA 400) at 25 °C in $CDCl_3$ using TMS as the internal standard. Chemical shifts are shown in δ. ^{13}C NMR spectra were recorded in a complete proton

decoupling environment. The chemical shift value is listed as δ_H and δ_C for ^1H and ^{13}C, respectively. Chiral GC analysis was performed in a Schimadzu autosampler with cyclodextrin columns as the chiral stationary phase (Fused silica capillary column, 30 m × 0.25 mm × 0.25 μm thick: β-Dex-120 and β-Dex-325 from SUPELCO, USA) using He as a carrier gas (detector temperature: 230 °C and injection temperature: 220 °C). Retention time was denoted as t_R and t_S for (*R*)- and (*S*)-enantiomers, respectively; values in parenthesis indicate the column temperature. Optical rotations were measured in a digital polarimeter HORIBA Sepa-3000 instrument. Mass spectra were measured in a QSTAR-XL (LC/MS) spectrometer (Applied Biosystems).

4.2. *PmHNL* isolation and assay

Details of the enzyme isolation and assay procedures are reported in our previous article.[5a] The protein content of *PmHNL* was measured using the Bradford method with a Bio-Rad protein assay kit using BSA as the standard.[15] Accordingly, the protein content of a crude extract of *PmHNL* was found to be roughly 10 mg/mL and activity was 120 U/mL. The enzyme is a flavoprotein (FAD containing), as evident by its yellow color and confirmed by absorption spectroscopy.

4.3. Preparative scale biphasic synthesis of cyanohydrins using *PmHNL*

Carbonyl compounds (5 g) were dissolved in diisopropylether (DIPE, 100 mL), saturated with 5 mL of 400 mM Na-citrate buffer (pH = 4.0). HNL solution was prepared by dissolving concentrated crude extract of *PmHNL* (100 mg of protein) in 10 mL of the above buffer. The enzyme solution was added to the reaction vessel followed by the addition of an appropriate cyanide source (acetonecyanohydrin: 1.5 equiv) then vigorously stirred until the desired conversion was achieved (by TLC at 25 °C). The reaction mixture was extracted with ether (3 × 50 mL) and dried over Na_2SO_4. The product cyanohydrins were purified by silica gel chromatography (hexane/ethyl acetate 8:2).

4.4. Preparation of racemic cyanohydrins as the GC standard

Following a common protocol, an aqueous solution of KCN (3 equiv) in water was added to a solution of carbonyl compounds (1 equiv) in acetic acid.[16] After completion of the reaction (by TLC), the reaction mixture was neutralized with aq $NaHCO_3$ solution then extracted with ether and dried over anhydrous Na_2SO_4. Evaporation and purification by column chromatography on silica resulted in a good yield of cyanohydrins. Racemic cyanohydrins obtained by this method were fully characterized by NMR spectroscopy and used as the GC standard.

4.5. Derivatization of cyanohydrins as their TMS ethers

Method a, for determination of enantioselectivity by chiral GC: Cyanohydrins synthesized enzymatically were dissolved in anhydrous DCM, then treated with excess imidazole and TMS-Cl at room temperature. After complete derivatization, the products were purified by passing through a small pad of silica gel and eluting with hexane/ETOAc (9:1). *Method b, synthesis of racemic cyanohydrin–TMS ethers as the GC Standard*: Aldehydes (1 equiv) were dissolved in anhydrous DCM, followed by the addition of a catalytic amount of anhydrous $ZnCl_2$ (0.03 equiv). The solution was stirred for 5 min at room temperature then trimethylsilylnitrile (1.5 equiv) was added to the reaction mixture. After complete conversion by TLC, the organic solvent was removed under vacuum and the product was purified as described earlier.

4.6. NMR values and GC retention times of aliphatic cyanohydrins

Spectral data (^1H NMR, ^{13}C NMR, and MS) for most compounds were in full agreement with the previously published data.[3,6–8,14,17,18] Except for compounds **17a**, **23a**, and **25a**, all cyanohydrins reported here have been reported elsewhere. The following referencing index will help the reader locate those compounds:

Compounds **1a**–**11a**, **12a**–**16a**, **20a**, **22a**, **24a**, **26a**, **30a**–**32a** can be found in Ref. 3b (and cross references cited there in); **18a**,[18] **19a**,[18c] **21a**,[18b] **27a**,[18d] **28a**,[18e] **29a**,[18d] **33a**,[18f] **34a**.[1f]

The GC retention times for cyanohydrins **1a**–**4a**, **6a**–**7a**, and **29a**–**30a** were reported in our previous article[5a] and hence are not given here.

4.6.1. (2*R*)-Hydroxy-4-methylpentanenitrile 5a. t_R = 26.1; t_S = 27.2 (125).

4.6.2. (2*R*)-Hydroxyoctanenitrile 8a. t_R = 46.8; t_S = 47.9 (100).

4.6.3. (2*R*)-Hydroxynonanenitrile 9a. t_R = 53.0; t_S = 54.3 (110).

4.6.4. (2*R*)-Hydroxydecanenitrile 10a. t_R = 57.3; t_S = 58.6 (120).

4.6.5. (2*R*)-Hydroxyundecanenitrile 11a. t_R = 59.8; t_S = 60.9 (130).

4.6.6. (2*R*)-2-Hydroxy-3-butenenitrile 12a. t_R = 45.2; t_S = 46.0 (90).

4.6.7. (2*R*)-2-Hydroxy-3-methyl-3-butenenitrile 13a. t_R = 32.2; t_S = 33.1 (110).

4.6.8. (2*R*,3*E*)-2-Hydroxy-3-pentenenitrile 14a. t_R = 61.7; t_S = 63.4 (100).

4.6.9. (2*R*,3*E*)-2-Hydroxy-3-methyl-3-pentenenitrile 15a. t_R = 34.1; t_S = 32.3 (125).

4.6.10. (2*R*)-2-Hydroxy-4-methyl-3-pentenenitrile 16a. t_R = 29.3; t_S = 26.7 (130).

4.6.11. (2*R*,3*E*)-3-Ethyl-2-hydroxy-3-pentenenitrile 17a. δ_H: 5.8 (q, $J = 7.3$ Hz, 1H), 4.82 (s, 1H), 3.85 (br s, 1H), 2.2 (m, 2H), 1.72 (d, $J = 7.2$ Hz, 3H), 1.0 (t, $J = 7.2$ Hz, 3H). δ_C: 136.2, 125.1, 118.9, 56.8, 29.8, 19.6, 11.8. $t_R = 41.4$; $t_S = 39.0$ (130). $[\alpha]_D^{25} = -32.1$ (c 1.0, $CHCl_3$).

ESIMS for $C_7H_{11}ON$ calcd 125.0913, obsd m/z 125.0916 (M^+).

4.6.12. (2*R*,3*E*)-2-Hydroxy-3-methyl-3-hexenenitrile 18a. δ_H: 5.68 (t, $J = 8.2$ Hz, 1H), 4.82 (s, 1H), 3.76 (br s, 1H), 2.08 (m, 2H), 1.8 (s, 3H), 1.0 (t, $J = 7.2$ Hz, 3H). δ_C: 134.2, 128.9, 118.6, 67.2, 21.2, 13.3, 11.8. $[\alpha]_D^{25} = -26.8$ (c 1.2, $CHCl_3$). $t_R = 30.7$; $t_S = 29.4$ (135).

4.6.13. (2*R*,3*E*)-3-Ethyl-2-hydroxy-3-heptenenitrile 19a. δ_H: 5.7 (m, 1H), 4.82 (s, 1H), 3.85 (br s, 1H), 2.2–1.98 (m, 4H), 1.52 (m, 2H), 1.0 (t, $J = 7.2$ Hz, 3H), 0.96 (t, $J = 7.2$ Hz, 3H). δ_C: 142.4, 122.5, 118.4, 68.5, 29.3, 23.4, 17.6, 14.2, 12.8. $t_R = 30.8$; $t_S = 30.0$ (150). $[\alpha]_D^{25} = -36.8$ (c 1.0, $CHCl_3$).

4.6.14. (2*R*,3*E*)-2-Hydroxy-3-heptenenitrile 20a. δ_H: 6.0 (m, 1H), 5.6 (dd, $J = 14.8$, 6.8 Hz, 1H), 4.92 (d, $J = 6.8$ Hz, 1H), 4.0 (br s, 1H), 2.08 (m, 2H), 1.45 (m, 2H), 0.92 (t, $J = 7.2$ Hz, 3H). δ_C: 137.7, 123.6, 118.6, 61.6, 33.9, 21.7, 13.4 $t_R = 42.9$; $t_S = 42.1$ (130).

4.6.15. (2*R*,3*E*)-2-Hydroxy-3-octenenitrile 21a. δ_H: 6.0 (m, 1H), 5.64 (dd, $J = 14.8$, 6.8 Hz, 1H), 4.92 (d, $J =$ 6.8 Hz, 1H), 4.0 (br s, 1H), 2.08 (m, 2H), 1.45–1.6 (m, 4H), 0.92 (t, $J = 7.2$ Hz, 3H). δ_C: 134.5, 126.4, 118.9, 66.6, 32.7, 32.2, 22.9, 14.2. $t_R = 38.9$; $t_S = 37.7$ (110).

4.6.16. (2*R*,3*E*)-2-Hydroxy-3-nonenenitrile 22a. δ_H: 6.1 (m, 1H), 5.46 (dd, $J = 15.2$, 6.9 Hz, 1H), 4.88 (d, $J =$ 6.9 Hz, 1H), 3.5 (br s, 1H), 2.1 (m, 2H), 1.2–1.5 (m, 6H), 0.95 (br t, 3H). δ_C: 137.8, 123.5, 118.6, 61.6, 32.2, 31.1, 28.0, 22.6, 13.8. $t_R = 42.5$; $t_S = 41.2$ (120).

4.6.17. (2*R*,3*E*)-2-Hydroxy-3-decenenitrile 23a. δ_H: 6.07 (m, 1H), 5.48 (dd, $J = 15.0$, 6.8 Hz, 1H), 4.87 (d, $J = 6.8$ Hz, 1H), 3.5 (br s, 1H), 2.1 (m, 2H), 1.2–1.5 (m, 8H), 0.92 (br t, 3H). δ_C: 137.8, 123.6, 118.6, 62.0, 31.8, 31.3, 28.9, 28.7, 22.4, 13.9. $t_R = 44.8$; $t_S = 43.7$ (130). ESIMS for $C_{10}H_{17}ON$ calcd 167.0913, obsd m/z 167.0918 (M^+).

4.6.18. (2*R*,3*E*,5*E*)-2-Hydroxy-3,5-heptadienenitrile 24a. $t_R = 32.1$; $t_S = 32.8$ (110).

4.6.19. (2*R*,3*E*,5*E*)-2-Hydroxy-3,5-octadienenitrile 25a. δ_H: 6.55 (dd, $J = 15.0$, 10.5 Hz, 1H), 6.08 (dd, $J = 15.0$, 10.5 Hz, 1H), 5.93 (dq, $J = 15.0$, 7.0 Hz, 1H), 5.65 (dd, $J = 15.0$, 7.0 Hz, 1H), 5.0 (d, $J = 7.0$ Hz, 1H), 2.88 (br s, 1H), 2.1 (q, $J = 7.0$ Hz, 2H), 1.02 (t, $J = 7.0$ Hz, 3H). δ_C: 141.6, 135.9, 126.8, 122.7, 118.3, 61.7, 25.7, 13.0.

ESIMS for $C_8H_{11}ON$ calcd 137.0913, obsd m/z 137.0918 (M^+).

$[\alpha]_D^{25} = -31.4$ (c 1.4, $CHCl_3$). $t_R = 46.8$; $t_S = 48.7$ (115).

4.6.20. (2*R*,3*E*)-2-Hydroxy-4,8-dimethyl-3,7-nonadienenitrile 26a. $t_R = 59.8$; $t_S = 59.3$ (120).

4.6.21. (2*R*)-2-Cyclobutyl-2-hydroxyacetonitrile 28a. δ_H: 4.28 (d, $J = 7.0$ Hz, 1H), 1.75–2.1 (m, 7H). δ_C: 119.2, 67.7, 35.2, 21.2, 19.0. $[\alpha]_D^{25} = +3.8$ (c 1.1, $CHCl_3$) $t_R = 25.5$; $t_S = 24.8$ (120).

4.6.22. (2*R*)-2-Cyclopentyl-2-hydroxyacetonitrile 29a. δ_H: 4.38 (d, $J = 7.2$ Hz, 1H), 2.8 (br s, 1H), 1.4–1.72 (m, 9H). δ_C: 118.5, 65.2, 35.9, 29.2, 27.3. $[\alpha]_D^{25} = +11.2$ (c 1.0, $CHCl_3$).

4.6.23. (2*R*)-2-Cyclohexenyl-2-hydroxyacetonitrile 31a. δ_H: 6.0 (m, 1H), 4.9 (s, 1H), 3.48 (br s, 1H), 2.1 (m, 2H), 1.5–1.8 (m, 6H). δ_C: 138.7, 129.4, 118.6, 65.3, 25.1, 24.7, 18.7, 18.0. ESIMS for $C_8H_{11}ON$ calcd 137.0913, obsd m/z 137.0906 (M^+). $[\alpha]_D^{25} = -16.7$ (c 1.5, $CHCl_3$). $t_R = 35.9$; $t_S = 37.6$ (160).

4.6.24. (2*R*)-2-(3-Cyclohexenyl)-2-hydroxyacetonitrile 32a. δ_H: 5.7 (m, 2H), 4.4 (d, $J = 7.1$ Hz, 1H), 2.1–1.91 (m, 4H), 1.7–1.48 (m, 3H). δ_C: 126.1, 125.8, 118.9, 65.7, 38.0, 26.6, 24.8, 23.4. $t_{major} = 48.4$, 49.6; $t_{minor} =$ 47.7, 49.3 (105).

4.6.25. (2*R*)-2-(Bicyclo[2.2.1]-hept-5-en-2-yl)-2-hydroxyacetonitrile 33a. δ_H: 5.65 (m, 2H), 4.24 (m, 1H), 2.26 (m, 2H), 1.94 (m, 1H), 1.72–1.58 (m, 3H), 1.34 (m, 1H). δ_C: 136.2, 135.8, 118.5, 63.4, 51.2, 42.7, 42.3, 32.8, 26.3. ESIMS for $C_9H_{11}ON$ calcd 149.0913, obsd m/z 149.0915 (M^+). $t_{major} = 43.9$, 49.4; $t_{minor} = 45.1$, 50.5 (160).

4.6.26. (2*R*)-2-Hydroxy-2-(6,6-dimethylbicyclo[3.1.1]hept-2-en-3-yl)acetonitrile 34a. δ_H: 5.9 (s, 1H), 4.85 (s, 1H), 2.65–2.1 (m, 6H), 1.6 (s, 3H), 1.3 (s, 3H). δ_C: 124.8, 123.6, 118.3, 63.4, 43.2, 40.0, 38.3, 34.1, 29.0, 25.8, 24.1. $[\alpha]_D^{25} = -21.1$ (c 1.4, $CHCl_3$). ESIMS for $C_{11}H_{15}ON$ calcd 177.0913, obsd m/z 177.0916 (M^+). $t_R = 36.3$; $t_S = 37.6$ (170).

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