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Enzymatic Synthesis of (*R*)-Cyanohydrins by Three (*R*)-Oxynitrilase Sources in Micro-aqueous Organic Medium

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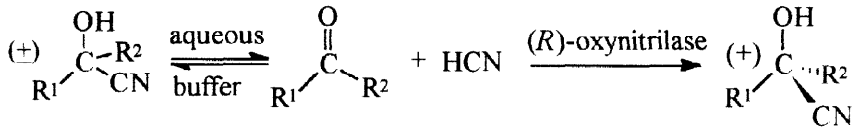
Abstract: The enantioselective synthesis of optically active (*R*)-cyanohydrins generated from several aromatic, heteroaromatic and aliphatic aldehydes and methyl ketones was carried out using almond, peach or loquat meal as (*R*)-oxynitrilase sources in diisopropyl ether under micro-aqueous conditions. The micro-aqueous reaction system, which is superior to the conventionally used water-organic biphase reaction system, performed well over the temperature range of 4°C to 30°C. © 1999 Published by Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The (*R*)-oxynitrilase (HNL, hydroxynitrile lyase, E.C.4.1.2.10), which catalyzes the reversible condensation of hydrogen cyanide with aldehydes, is a useful and promising enzyme for biotransformation. The resulting optically active cyanohydrins are expedient starting materials for the preparation of several important classes of compounds such as α-hydroxyketones, α-hydroxyacids, β-aminoalcohols as well as amino-nitriles and aziridines.[1,2] It has so far been reported that the (*R*)-oxynitrilase-catalyzed reactions were carried out with purified enzymes[1,3,4] or crude enzyme preparations[5-10] from almond, flax, plum, cherry, apricot, *etc*. In general, aqueous buffer media were chosen as favorable reaction conditions.[5,11] However, due to a competing nonenzymatic reaction of the substrate with cyanide simultaneously, it is necessary to establish usage conditions. Otherwise the reaction fails to achieve high optical purities in aqueous media. In addition, the enantiomeric purities of the enzymatic products could be further compromised by their racemization in the aqueous buffer during the course of the reaction.



To diminish the competing non-enzymatic formation of a racemic mixture, the reaction was usually performed in a weak acidic buffer solution (PH 3.5-5.5)[5,11] or in a suitable biphasic water-water immiscible organic solvent system at lower temperatures (0-4°C).[3,4,6-10]

We[12] have disclosed that at room temperature the nonenzymatic chemical addition was still observed in the water-organic biphasic reaction system though the volume of aqueous phase was relatively small. However, it was not a problem over a wide range of temperatures when the reactions were carried out under the

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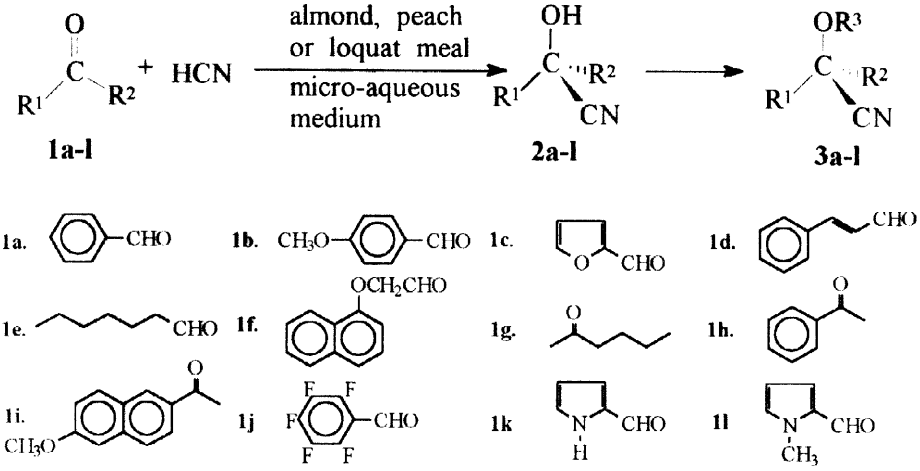
micro-aqueous conditions. In that case the organic phase served as a big reservoir of substrates and products, where the catalytic enzyme meal which retained essential water spread homogeneously and acted as a highly effective mode of transfer. Higher yields and enantiomeric selectivities were achieved as the nonenzymatic addition and decomposition were almost suppressed.

RESULTS AND DISCUSSION

In this paper, the almond (*Prunus armeniaca L.*), peach (*Prunus persica L.*) and loquat (*Eriobotrya L.*) kernels were used as (*R*)-oxynitrilase sources for the synthesis of (*R*)-cyanohydrins from aliphatic and aromatic aldehyde as well as methyl ketone under micro-aqueous conditions in high enantioselectivities. This procedure did not require an aqueous work-up; the substrate was treated with the enzyme meal in single organic phase. After completion of the reaction, the enzymes were recovered for re-use by simple filtering. Upon removal of the solvent and excess of HCN under reduced pressure, the crude products were obtained. Besides, this procedure worked well at the range of temperatures from 4°C to 30°C with various aldehydes and ketones as the substrate to give the desired products in high yields and enantiopurities on most occasions. For the determination of optical activities, the cyanohydrin products were acetylated or *p*-chlorobenzoylated for chiral HPLC analysis.



Scheme 1. Substrates investigated for (*R*)-cyanohydrination under micro-aqueous medium conditions.

The results of the enzymatic cyanohydrination performed both in biphasic water-organic solvent (system I) and in micro-aqueous organic solvent (system II) are shown in Table 1, and in Table 2 for comparison. At lower temperature (4°C), the synthesis of (*R*)-mandelonitrile **2a** was accomplished in quantitative yields and excellent enantiomeric purities (>99% ee) both in system I (Entry 1,2) and in system II (Entry 9). For almond enzyme, with an increase of the temperature, in system I as Table 1 shows, the yields and ees of **2a** decreased to 92% and 97.5%, respectively, in IPE/Buffer at 12°C (Entry 4). The yields and ees were further decreased to 65% and 92% in EA/Buffer (Entry 5) as well as to 77% and 93.9% in IPE/Buffer (Entry 6) at 30°C, respectively. In system II as Table 2 shows, **2a** was obtained in higher yield and ee, exemplified by 100% yield and >99% ee (Entry 10,11) at 12°C, 98% yield and 98.2% ee in EA (Entry 12) as well as 98% yield and

97% ee in IPE (Entry 13) at 30°C, respectively. For loquat enzyme, the yield and enantiopurity for **2a** decreased to 76% and 81% respectively in system I (Entry 7) with an increase in temperature to 30°C. Under the same condition, the system II method afforded higher yield (95%) and enantioselectivity (97%) (Entry 15) which matched the same level as that at 4 °C (Entry 14). Moreover, in system II (*S*)-(+)-2-(2-furyl)-2-hydroxyacetonitile **2c** was obtained equally well both at 4 °C (Entry 16) and at 30°C (Entry 17), while in system I it was only obtained with 70% yield and 73% ee at 30°C (Entry 8).

These results indicated that a lower temperature was necessary in system I to keep the concurring non-enzymatic reaction at as low a level as possible for achieving better results. At higher temperatures such as

Table 1. Investigations for the enantioselective syntheses of (*R*)-cyanohydrins in water-water immisible organic biphase

Entry	Substrate	Source of Enzyme	Solvent	Temp.(°C)	Time (h)	Yield(%)[a]	ee(%)[b]
1	**1a**	Almond	EA+Buffer	4	48	100	>99
2			IPE+Buffer	4	48	100	>99
3			EA+Buffer	12	48	94	>99
4			IPE+Buffer	12	48	92	97.5
5			EA+Buffer	30	24	65	92
6			IPE+Buffer	30	24	77	93.9
7		Loquat	IPE+Buffer	30	24	76	81
8	**1c**	Almond	IPE+Buffer	30	12	70	73

EA: Ethyl acetate; IPE: Diisopropyl ether; Buffer: 5%(v/v) 0.02M citrate buffer (pH 5.5). a) Isolated yield after column chromatography; b) Determined by HPLC analysis on CHIRALPAK AD column as the *O*-protected derivatives.

Table 2. Investigations for the enantioselective syntheses of (*R*)-cyanohydrins in micro-aqueous organic medium.

Entry	Substrate	Source of Enzyme	Solvent	Temp.(°C)	Time (h)	Yield(%)[a]	e.e (%)[b]
9	**1a**	Almond	IPE	4	48	100	>99
10			EA	12	48	100	>99
11			IPE	12	48	100	>99
12			EA	30	24	98	98.2
13			IPE	30	24	98	97
14		Loquat	IPE	4	48	99	99
15			IPE	30	48	>95	>97
16	**1c**	Almond	IPE	4	24	100	99
17			IPE	30	12	90	95

EA (water content 0.20-1.52% v/v), IPE (water content 0.14-0.32% v/v). a) Isolated yield after column chromatography; b) Determined by HPLC analysis on CHIRALPAK AD column as the *O*-protected derivatives.

30°C, the rates of chemical addition and decomposition were enhanced, resulting in a decrease of the chemical yields and ees. The rates of decomposition of racemic mandelonitrile both in citrate buffer and in micro-aqueous medium solutions were monitored by determining the intensity of the absorption (wave length 249.6 nm) of PhCHO at 25°C for comparison. The result is, the concentration of PhCHO increased steadily in citrate buffer. In contrast, the (±)-mandelonitrile was stable in micro-aqueous organic medium (EA or IPE) and no PhCHO resulted. This result proved that the nonenzymatic addition and decomposition that existed in water-organic biphase were suppressed strongly in micro-aqueous media.

The almond enzyme meal was recovered and used repeatedly for twenty times in 15 days with **1a** as substrate in the procedure for system II. No decrease in conversions and ees was found under such micro-aqueous condition.

To examine the reaction scope, various substrates (Scheme 1) were investigated under micro-aqueous conditions using (*R*)-oxynitrilase prepared from three kinds of enzyme sources (Table 3). Clearly, the almond and peach meal exhibited similar activities in the catalytic reactions, affecting the substrates including aromatic, heteroaromatic, and aliphatic aldehydes as well as methyl ketones. In the meanwhile, the loquat enzyme did not accept an aliphatic aldehyde (Entry 35 and 36) (Trimethylacetaldehyde was found to be not accepted yet by the enzyme) or ketones (Entry 41 and 44). The almond enzyme is more favorable than loquat enzyme in most cases.

High reactivities and enantioselectivities were exhibited by aldehydes **1a** (Entry 9, 13-15 in table 2 and Entry 18), **1b** (Entry 19-23) and **1c** (Entry 16-17 in table 2 and Entry 24-25) under micro-aqueous medium both at 4°C and at 30°C using almond, peach and loquat as enzyme resources. While it was reported[5] that α,β-unsaturated (*E*)-cinnamaldehyde **1d** was unaffected by the enzyme in aqueous medium, it could be converted into the corresponding (*R*)-cyanohydrin **2d** with moderate yields and enantiopurities in micro-aqueous conditions (Entry 26-31). The enantioselectivity of the cyanohydrin **2d** by using peach enzyme (69.3% ee, Entry 30) is higher than that by using almond enzyme (49% ee, Entry 28). In that regard a higher temperature was needed in order to increase the chemical yields and once again the ee values did not decline compared with those at a lower temperature. The aliphatic aldehyde **1e** only led to product **2e** in moderate enantioselectivities (Entry 32-34) at 4°C or 30°C using almond or peach meal since the side reactions happened. The cyanohydrin biotransformed **1f** was found to be racemic (97% yield, Entry 37). Noteworthy was the fact that the parallel experiment of **1f** without using the enzyme showed no product at all (Entry 38). Griengl,[13] also found that the cyanohydrinations of phenoxy- and benzyloxyacetaldehyde yielded the racemate with (*S*)-hydroxynitrile lyase from *Hevea brasiliensis* in citrate buffer solution, and the same observation of phenoxyacetaldehyde was seen using a (*R*)-hydroxynitrile lyase from almond in ethanol/acetate buffer,[5] we suggested that there may be an interaction between the α-oxygen atom of the aldehyde **1f** and the active site of the enzyme, which resulted in the lost of stereoselectivity.

The methyl ketone **1g** was transferred to **2g** with 98.6% ee using almond (Entry 39) and 98.2% ee using peach (Entry 40) at 25°C. The reaction of aromatic methyl ketones under the same condition appeared to be taking place much slowly. **1h** gave the desired product in only 33% yield and 78% ee using almond enzyme (Entry 42) as well as 22% yield and 72% ee using peach enzyme (Entry 43) at 25°C after 120hrs. Whereas, **1i** did not offer any product apart from the unchanged substrate (Entry 45). The cyanohydrin **2j** could be prepared

in 90% yield from pentafluorobenzaldehyde; however, like **1f**, the product was turn to racemate (Entry 46). The nitrogen containing and *N*-protected heteroaromatic aldehyde pyrrol-2-aldehyde **1k** (Entry 47) and *N*-methyl-pyrrol-2-aldehyde **1l** (Entry 48) were not affected by almond enzyme treatment for 2 days at 20°C.

Table 3. Synthesis of **2a-l** (*R*)-cyanohydrins in micro-aqueous diisopropyl ether in the presence of various oxynitrilase source.

Entry	Substrate	Source of Enzyme	Temp. (°C)	Time (h)	Yield(%)[a]	e.e.(%)[b]
18	**1a**	Peach	4	48	99	99
19	**1b**	Almond	4	48	48	95.3
20			30	48	60	92.4
21		Peach	30	48	56	91
22		Loquat	4	48	12	90.5
23			30	48	20	74.5
24	**1c**	Peach	4	24	93	98.2[c]
25		Loquat	4	24	50	91[c]
26	**1d**	Almond	4	48	9.6	57
27			30	24	36.5	51.5
28			30	48	58	49
29		Peach	4	48	7.8	68
30			30	24	30	69.3
31		Loquat	4	48	3.3	52.2
32	**1e**	Almond	4	45	46	41[d]
33			25	48	80	67.1[d]
34		Peach	4	45	39	58.1[d]
35		Loquat	4	45	6.6	0[d]
36			30	45	0	0
37	**1f**	Almond	15	84	97	0[e]
38		No enzyme	15	84	0	0
39	**1g**	Almond	25	45	68	98.6[d]
40		Peach	25	45	42	98.2[d]
41		Loquat	25	45	0	0
42	**1h**	Almond	25	120	33[f]	78.1
43		Peach	25	120	22[f]	72
44		Loquat	25	120	0	0
45	**1i**	Almond	30	48	0	0
46	**1j**	Almond	12	48	90	0
47	**1k**	Almond	20	48	0	0
48	**1l**	Almond	20	48	0	0

a) Isolated yield after column chromatography; b) Determined by chiral HPLC after acetylation; c) (*S*)-configuration was assigned according to Cahn-Ingold-Prelog; d) Determined by chiral HPLC after *p*-chlorobenzoylation with *p*-chlorobenzoyl chloride; e) As (*S*)-(-)-MTPA derivatives; f) Based on acetate obtained.

CONCLUSION

In summary, we have succeeded in developing a convenient and reliable way to prepare (*R*)-cyanohydrins in high enantioselectivity by employing the crude (*R*)-oxynitrilase from almond, peach and loquat in micro-aqueous organic medium at temperatures ranging from 4°C to 30°C. The characteristic feature is that the micro-aqueous reaction system, which is superior to the conventionally used water-organic biphasic reaction system, performed well even at 30°C where the nonenzymatic addition and decomposition were almost suppressed. The accessible substrates by peach enzyme are very similar to those by almond enzyme. However, the substrate scope of loquat enzyme, unlike almond or peach enzyme, is restricted to aromatic and heteroaromatic aldehydes. For α,β-unsaturated (*E*)-cinnamaldehyde **1d**, the enantioselectivity of the cyanohydrin **2d** by using peach enzyme is higher than that by using almond enzyme.

EXPERIMENTAL

The almonds were purchased from a local medical store, the kernels of peach and loquat were collected from mature garden fruits. After being granulated in a homogenizer, the preparations were defatted four times with ethyl acetate. The defatted meal (water contents in the meals were 8-10% w/w) was stored in a refrigerator at 4°C.

Diisopropyl ether was distilled before use. Commercially available substrates were distilled before use. Hydrogen cyanide in diisopropyl ether or ethyl acetate solution was prepared according to the literature method[4] and was stored in a freezer. Racemic aldehyde and ketone cyanohydrins were prepared from the corresponding aldehydes and ketones with NaCN according to the known method.[14]

^1H, ^{13}C and ^{19}F NMR spectra were recorded at 300 MHz, 75 MHz and 282 MHz respectively on a Bruker AMX-300 instrument in $CDCl_3$ using tetramethylsilane as an internal standard. IR spectra were recorded on a Digibal FTIR instrument. EI-MS spectra on a HP-5989A instrument. Optical rotations were measured using a Perkin-Elmer 241 MC polarimeter. Elemental analyses were carried out on a Heraeus Rapid - CHNO elemental analyzer. Fluorine contents were determined by Th salt titration. Water contents were determined on a METTLER DL35 Karl-Fischer titrator. UV spectra were recorded on a Beckman DU-70 spectrometer. The enantiomeric purities of the cyanohydrin were determined after acetylation or *p*-chlorobenzoylation with the chiral HPLC resolution on CHIRALPAK AD column.

Procedure for system I (in water-organic biphasic medium):

In a typical experiment, the enzyme preparation (500 mg) was swollen with 0.53 ml of a 0.02 M citrate buffer (PH 5.5) for 10 min., freshly distilled aldehyde (2.5 mmol) and 1.5 eq. HCN in 10 ml diisopropyl ether were mixed. The resulting aqueous pulp of almond meal in organic solvent was stirred at the temperature and the time indicated in Table 1.

Procedure for system II (in micro-aqueous organic medium):

In a typical experiment, the enzyme preparation (500 mg), freshly distilled aldehyde (2.5 mmol) and 1.5 eq. HCN in 10 ml diisopropyl ether (the solution was dried over Na_2SO_4 before used, water content 0.32% v/v)

were mixed. The fine enzyme powder spread homogeneously in organic solvent when the mixture was stirred at the temperature and the time indicated in Tables 2 and 3.

Upon removal of the crude enzyme by filtration, the filtrate was concentrated under reduced pressure, and the crude cyanohydrin was purified by column chromatography on silica gel. The yields were for isolated cyanohydrins. After measuring the optical rotation of the free cyanohydrins, the compounds were converted directly into the corresponding *O*-protected derivatives at room temperature. The organic layer was washed subsequently with saturated $CuSO_4$, H_2O, brine solution, and afterwards drying with anhydrous Na_2SO_4. Upon removal of the solvent under reduced pressure, the crude residues were purified by flash chromatography to yield the *O*-protected cyanohydrins. The enantiomeric purity of the cyanohydrins were determined with the chiral HPLC resolution on CHIRALPAK AD column as the corresponding *O*-protected derivatives

(*R*)-(+)-2-Hydroxy-2-phenylacetonitrile (2a). $[\alpha]_D^{20}$ +47.5 (c 1.89, $CHCl_3$), e.e. >99%; Lit.[5]: $[\alpha]_D^{20}$ +45 (c 1, $CHCl_3$), e.e. >99%. Isolated yield (after chromatography): 100%. ^{1}H NMR: δ, 3.70 (s, 1H, OH); 5.50 (s, 1H, CH); 7.50 (br s, 5H, Ar-H). IR: 3414, 3066, 3036, 2250, 1495, 1456, 1406, 1196, 1043, 765, 701cm^{-1}. MS: m/z (rel. intensity %): 133(M^+, 78), 132(M^+-1, 50), 116(29), 115(43), 106(38), 105(92),91(14), 77(100), 57(9), 51(45), 43(21).

(*R*)-(+)-2-Acetoxy-2-phenylacetonitrile (3a). $[\alpha]_D^{20}$+4.5 (c 1.8, $CHCl_3$), e.e. >99%; Lit.[13]: $[\alpha]_D^{20}$ -7.24 (c 2.3, $CHCl_3$) for the (*S*)-isomer, e.e. >99%. Isolated yield (after chromatography): 100%. ^{1}H NMR: δ, 1.87 (s, 3H, CH_3CO); 6.41(s, 1H, CH); 7.47(m, 5H, Ar-H). IR: 3039, 2946, 1756, 1373, 1216, 1025, 759, 697cm^{-1}. MS: m/z (rel. intensity %): 175(M^+, 11), 133(100), 116(67), 115(76), 105(43), 89(21), 77(30), 63(11), 51(17), 43(59).

(*R*)-(+)-2-Hydroxy-2-(4-methoxyphenyl)acetonitrile (2b). Colorless solid, $[\alpha]_D^{18}$ + 48.5 (c 0.89, $CHCl_3$), e.e. 92.4%; Lit.[5]: $[\alpha]_D^{20}$ +49 (c 1, $CHCl_3$), e.e. 99%. Isolated yield (after chromatography): 60%. ^{1}H NMR: δ, 3.18 (s, 1H, OH); 3.82 (s, 3H, CH_3O); 5.46 (s, 1H, CH); 6.94 (d, 2H, J=8.7 Hz, Ar-H); 7.43 (d, 2H, J=8.7 Hz, Ar-H). IR: 3398, 2248, 1613, 1515, 1023, 823cm^{-1}. MS: m/z (rel. intensity %): 163(M^+, 2), 137(11), 136(91), 135(100), 107(15), 92(14), 77(25), 63(9), 51(5).

(*R*)-(-)-2-Acetoxy-2-(4-methoxyphenyl)acetonitrile (3b). $[\alpha]_D^{22}$ -18.7 (c 0.86, $CHCl_3$), e.e. 95.3%; Lit.[13]: $[\alpha]_D^{20}$ +19.0 (c 1.55, $CHCl_3$) for the (*S*)-isomer, e.e. 95%. Isolated yield (after chromatography): 100%. ^{1}H NMR: δ, 2.14(s, 3H, CH_3CO); 3.83(s, 3H, OCH_3); 6.35(s, 1H, CH); 6.95(d, 2H, J=8.7 Hz, Ar-H); 7.45 (d, 2H, J=8.7 Hz, Ar-H). IR: 2950, 2842, 1755, 1612, 1516, 1256, 1217, 1179, 1029, 962, 831, 570cm^{-1}. MS: m/z (rel. intensity %): 205(M^+, 33), 163(13),146(100), 145(40), 137(17), 135(13), 116(6), 103(5), 91(7), 76(6), 63(4), 51(2), 43(18).

(*S*)-(+)-2-Hydroxy-2-(2-furyl)acetonitrile (2c). $[\alpha]_D^{21}$ +50.0 (c 1.60, $CHCl_3$), e.e. 99%; Lit.[15]: $[\alpha]_D^{25}$ +14 (c 1.8698, $CHCl_3$), e.e. 79%. Isolated yield (after chromatography): 100%. ^{1}H NMR: δ, 3.17 (br s, 1H, OH); 5.55(s, 1H, CH); 6.43(dd, 1H, J=1.9Hz, J=3.3 Hz, Ar-H); 6.61(m, 1H, Ar-H); 7.49(m, 1H, Ar-H). IR: 3403, 2256, 1707, 1500, 1400, 1269, 1234, 1149, 1030, 750cm^{-1}. MS: m/z (rel. intensity %): 123(M^+, 53), 106(64), 97(27), 96(87), 95(100), 91(70), 77(28), 68(42), 57(76), 43(52).

(*S*)-(-)-2-Acetoxy-2-(2-furyl)acetonitrile (3c). $[\alpha]_D^{20}$ -26.3(c 1.40, CHCl3), e.e. 99%; Lit.[13]: $[\alpha]_D^{20}$ +24.3 (c 1.6, CHCl3) for the (*R*)-isomer, e.e. 98%. Isolated yield (after chromatography): 100%. ^{1}H NMR: δ, 2.16(s, 3H, CH_3CO); 6.44(dd, 1H, J=1.97Hz,J=3.32 Hz, Ar-H); 6.47(s, 1H, CH); 6.68(d, 1H, J=3.32 Hz, Ar-H); 7.51(d, 1H, J=1.23 Hz, Ar-H). IR: 3103, 2950, 1755, 1373, 1215, 1015, 755cm^{-1}. MS: m/z (rel. intensity %): 165(M^+, 17),

147(76), 133(73), 123(34), 116(50), 115(58), 106(71), 105(62), 95(10), 89(17), 77(69), 63(11), 51(31), 43(100).

(*R*)-(+)-2-Hydroxy-4-phenyl-(*E*)-but-3-enenitrile (2d). White solid, $[\alpha]_D^{13}$ +26.1 (c 0.78, $CHCl_3$), e.e. 69.3%; Lit.[16]: $[\alpha]_D^{26}$ -19.0 (c 1, $CHCl_3$) for the (*S*)-isomer, e.e. 62%. Isolated yield (after chromatography): 30%. ^1H NMR: δ 3.00(d, 1H, J=7 Hz,OH); 5.17(t, 1H, J=5.9 Hz, CH); 6.27(dd, 1H, J=5.9 Hz, J=15.9 Hz, =CH); 6.92(d, 1H, J=15.9 Hz, =CH); 7.33-7.44(m, 5H, Ar-H). IR: 3358, 3030, 2253, 1654, 1492, 1450, 1415, 1300, 1088, 1024, 976, 925, 756, 695cm^{-1}. MS: m/z (rel. intensity %): 159(M^+, 62), 142(18), 133(96), 132(44), 131(100), 130(78), 115(35), 105(26), 104(35), 103(44), 91(35), 78(25), 77(34), 63(15), 51(24), 43(0.7).

(*R*)-(-)-2-Acetoxy-4-phenyl-(*E*)-but-3-enenitrile (3d). $[\alpha]_D^{22}$ -22.73 (c 0.70, $CHCl_3$), e.e. 69.3%; Isolated yield (after chromatography): 100%. ^1H NMR: δ, 2.18(s, 3H, CH_3CO); 6.03(d, 1H, J=7 Hz, CH); 6.20(dd, 1H, J=7 Hz, J=15.8 Hz, =CH); 6.98(d, 1H, J=15.7 Hz, =CH); 7.35-7.45(m, 5H, Ar-H). IR: 3062, 3030, 2941, 1753, 1656, 1498, 1451, 1372, 1217, 1022, 968, 750, 693cm^{-1}. MS: m/z (rel. intensity %): 201(M^+, 6), 183(2), 159(100), 142(84), 141(76), 140(65), 133(19),131(36), 115(74), 103(18), 89(12), 77(21), 63(12), 51(15), 43(89).

(R)-(+)-2-[(tert-Butyldimethylsilyl)oxy]-4-phenyl-(*E*)-but-3-enenitrile (3d'). $[\alpha]_D^{15}$ +6.62 (c 0.94, $CHCl_3$), e.e. 69.3%; Lit.[13]: $[\alpha]_D^{20}$ -6.27 (c 0.35, $CHCl_3$) for the (*S*)-isomer, e.e. 95%. Isolated yield (after chromatography): 100%. ^1H NMR: δ, 0.19 (s, 3H, CH_3); 0.22 (s, 3H, CH_3); 0.94(s, 9H, $C(CH_3)_3$); 5.13 (dd, 1H, J=5.8 Hz, J=1.4 Hz, CHCN); 6.18(dd, 1H, J=15.7 Hz, J=5.8 Hz, C=CH-C-CN); 6.81(dd, 1H, J=15.8 Hz, J=1.2Hz, PhCH=C); 7.32-7.41 (m, 5H, Ar-H). ^{13}C NMR: δ, 135.23 (C-Ar);133.75(C=C); 128.83, 127.04 (C-Ar); 123.85(C=C); 118.53(CN); 62.74(CH); 25.64($C(CH_3)_3$); 18.27(C-Si); -4.90(CH_3-Si). IR: 2945, 2860, 1472, 1256, 1110, 967, 839, 782, 693cm^{-1}. MS: m/z (rel. intensity %): 273(M^+, 2.5), 258(2), 247(8), 217(30), 216(84), 190(27), 189(100), 142(38), 115(64), 103(3), 84(3), 75(27), 57(7), 45(3).

(*R*)-(+)-2-Hydroxy-octanenitrile (2e). $[\alpha]_D^{25}$+7.5 (c 0.75, $CHCl_3$), e.e. 67.1%; Isolated yield (after chromatography): 80%. ^1H NMR: δ, 0.88(t, 3H, J=7 Hz, CH_3); 1.23-1.62(m, 8H, 4xCH_2); 1.84(q, 2H, J=7 Hz, CH_2); 2.98(br s, 1H, OH); 4.47(t, 1H, J=7 Hz, CH). IR: 3442, 2931, 2861, 2249, 1467, 1071cm^{-1}. MS: m/z (rel. intensity %): 142(M^++1, 0.6), 141(M^+, 0.3), 140(M^+-1, 0.5), 124(2), 123(0.9), 122(5), 115(52), 108(6), 99(29), 97(66), 85(25), 81(32), 70(59), 68(34), 57(58), 55(68), 43(100).

(*R*)-(+)-2-(*p*-Chlorophenzoyloxy)-octanenitrile (3e). $[\alpha]_D^{22}$+16.5 (c 0.27, $CHCl_3$), e.e. 67.1%; Isolated yield (after chromatography): 100%. ^1H NMR: δ, 0.89(t, 3H, J=6.6 Hz, CH_3); 1.25-1.49(m, 6H, 4,5,6-H), 1.54-1.62(m, 2H, 3-H); 2.00-2.07(m, 2H, 2-H); 5.56(t, 1H, J=6.8 Hz, CH); 7.45(d, 2H, J=8.6 Hz, Ar-H), 7.98(d, 2H, J=8.6 Hz, Ar-H). IR: 2931, 2860, 1735, 1595, 1489, 1467, 1403, 1263, 1094, 849cm^{-1}. MS: m/z (rel. intensity %): 282(M^++2, 2), 281(M^++1, 0.8), 280(M^+, 4), 156(40), 141(39), 139(100), 123(3), 111(34), 95(9), 81(34), 75(20), 70(7), 55(21), 43(14).

(±)-2-Hydroxy-3-naphthoxypropanenitrile (2f). Isolated yield (after chromatography): 97%. ^1H NMR: δ, 3.47(br s, 1H, OH); 4.35(m, 2H, CH_2); 4.90(t, 1H, J=4.6 Hz, CH); 6.77(d, 1H, J=7.6 Hz, Ar-H); 7.37(t, 1H, J=8.0 Hz, Ar-H); 7.49-7.55(m, 3H, Ar-H); 8.25-8.29(m, 1H, Ar-H). IR: 3453, 3054, 2945, 2251, 1597, 1581, 1508cm^{-1}, 1400, 1269, 1242, 1100, 794, 770cm^{-1}. MS: m/z (rel. intensity %): 213(M^+, 33), 186(88), 157(50), 144(64), 143(49), 127(64), 115(100), 101(7), 89(11), 77(11), 63(8), 51(5), 43(3). Anal. calcd for $C_{13}H_{11}NO_2$: C, 73.21; H, 5.20; N, 6.57. Found: C, 73.19; H, 5.19; N, 6.18.

(*R*)-2-Hydroxy-2-methylhexanenitrile (2g). $[\alpha]_D^{22}$ +2.2 (c 1.33, $CHCl_3$), e.e. 98.6%; Isolated yield (after chromatography): 68%. ^1H NMR: δ, 0.94(t, 3H, J=7.1 Hz, 6-H); 1.24(t, 2H, J=7 Hz, 5-H); 1.35-1.51(m, 2H, 4-H); 1.59(s, 3H, 1-H); 1.73-1.79(m, 2H, 3-H). IR: 3445, 2961, 2938, 2875, 2242, 1465, 1379, 1172, 1143, 1063, 957, 887cm^{-1}. MS: m/z (rel. intensity %): 127(M^+, 0.7), 121(0.2), 110(5), 102(13), 101(100), 94(1), 85(3), 83(5), 71(13), 68(6), 57(8),43(14).

(*R*)-(-)-2-(*p*-Clorobenzoyloxy)-2-methylhexanenitrile (3g). $[\alpha]_D^{18}$-9.4 (c 0.21, $CHCl_3$), e.e. 98.6%; Isolated yield (after chromatography): 77%. ^1H NMR: δ, 0.97(t, 3H, J=7.2 Hz, 6-H); 1.37-1.66(m, 4H, 4,5-H), 1.87(s, 3H, 1-H); 1.98-2.17(m, 2H, 3-H); 7.44(d, 2H, J=8.5 Hz, Ar-H); 7.94(d, 2H, J=8.5 Hz, Ar-H). IR: 2960, 2930, 2860, 1734, 1594, 1488,1402, 1276, 1093, 849cm^{-1}. MS: m/z (rel. intensity %): 268(M^++2, 5), 267(M^++1, 2), 266(M^+, 10), 239(2), 209(0.7), 167(0.6), 156(19), 149(2), 141(47), 139(100), 126(4), 111(24), 94(5), 83(2), 75(18), 68(7), 50(6), 43(3).

(*R*)-(+)-2-Hydroxy-2-phenylpropanenitrile (2h). $[\alpha]_D^{17}$ +4.3 (c 0.47, $CHCl_3$), e.e. 78.1%; Isolated yield (after chromatography): 33%. ^1H NMR: δ, 1.84 (s, 3H, CH_3); 2.97(s, 1H, OH); 7.33-7.41(m, 3H, Ar-H); 7.53-7.56(m, 2H, Ar-H). IR: 3421, 2927, 2856, 2250, 1494, 1451, 1371, 1226, 1101, 764, 699cm^{-1}. MS: m/z (rel. intensity %):149(M^++2, 9), 148(M^++1, 5), 147(M^+, 36), 132(100), 121(10), 105(55), 91(5), 77(34), 63(4), 51(20), 43(14).

(*R*)-(-)-2-Acetoxy-2-phenylpropanenitrile (3h). $[\alpha]_D^{22}$ -15.2 (c 0.34, $CHCl_3$), e.e. 78.1%; ^1H NMR: δ, 1.99(s, 3H, CH_3); 2.14(s, 3H, CH_3CO); 7.35-7.54(m, 5H, Ar-H). IR: 3066, 3034, 2999, 2941, 1760, 1494, 1451, 1371, 1221, 1080, 765, 699cm^{-1}. MS: m/z (rel. intensity %): 190(M^++1, 2), 189(M^+, 0.7), 163(5), 147(100), 146(38), 130(73), 121(10), 116(4), 105(23), 103(39), 91(5), 77(37), 63(6), 51(20), 43(74).

(±)-2-Hydroxy-2-pentaflorinophenylacetonitrile (2j). Isolated yield (after chromatography): 90%. ^1H NMR: δ, 3.95(s, 1H, OH); 5.83(s, 1H, CH). ^{19}F NMR: δ, -82.5 (m, 2F, F-Ar); -72.3 (t, 1F, F-Ar); -64.7 (m, 2F, F-Ar). IR: 3494, 2953, 2266, 1660, 1515, 1137, 1052, 1002, 894, 787, 658cm^{-1}. MS: m/z (rel. intensity %): 224(M^++1, 12), 223(M^+, 100), 206(78), 203(31), 197(43), 196(32), 195(43), 177(41), 168(20), 167(21), 149(10), 117(23), 99(19), 56(7), 43(0.8). Anal. calcd for $C_8H_2F_5NO$: C, 43.05; H, 0.90; N, 6.28. Found: C, 42.97; H, 0.82; N, 6.12.

(±)-2-Acetoxy-2-pentaflorinophenylacetonitrile (3j). Isolated yield (after chromatography): 100%. ^1H NMR: δ, 2.19(s, 1H, CH_3); 6.68(s, 1H, CH). ^{19}F NMR: δ, -82.2 (m, 2F, F-Ar); -70.9(m, 1F, F-Ar); -62.1 (m, 2F, F-Ar). IR: 2963, 1770, 1659, 1515, 1211, 1030, 908cm^{-1}. MS: m/z (rel. intensity %): 266(M^++1, 6), 265(M^+, 4), 239(2), 223(99), 206(100), 195(12), 179(28), 167(7), 155(7), 117(9), 106(5), 93(6), 69(4), 43(75). Anal. calcd for $C_{10}H_4F_5NO_2$: F, 35.83. Found: F, 35.62.

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