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Novel (*R*)-oxynitrilase sources for the synthesis of (*R*)-cyanohydrins in diisopropyl ether

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Abstract: Apple, apricot, cherry and plum meal were prepared from the seeds or kernels of mature garden fruits. The preparations as well as almond meal were used as the source of (*R*)-oxynitrilase for the synthesis of aliphatic and aromatic cyanohydrins in diisopropyl ether in the presence of 0.1 M tartrate buffer [2% (v/v), pH 5.4]. Apple meal as the most favourable of the enzyme preparations accepts also sterically hindered aldehydes (*e.g.*, pivalaldehyde) as substrates, leading to (*R*)-cyanohydrins with high enantiopurity (usually ee>90%). © 1997 Elsevier Science Ltd

Hydrogen cyanide adds to many aldehydes and ketones to give hydroxynitriles, usually called cyanohydrins. These products are useful intermediates for organic synthesis and usually contain a new stereogenic centre, cyanohydrins obtained from symmetric ketones (*e.g.*, acetone) being exceptions. The methods of preparing those optically active addition products include metal complexes, synthetic dipeptides and oxynitrilases (hydroxynitrile lyases) as chiral catalysts.[1–9] Enzymatic addition of hydrogen cyanide always competes with the chemical addition. In order to suppress the chemical reaction which leads to racemic products the enzymatic reactions are preferably performed in organic media such as in diisopropyl ether in the presence of small amounts of a buffer.[7–9]

(*R*)-Oxynitrilase from almonds [EC 4.1.2.10] and (*S*)-oxynitrilase from *Sorghum bicolor* [EC 4.1.2.11] are commercial enzymes and as such widely used for the preparation of the (*R*)- and (*S*)-enantiomers of cyanohydrins, respectively. It was previously shown that almond meal and dechlorophylled shoots of *Sorghum bicolor* can be used instead of the isolated enzymes,[10–13] which considerably reduces the costs. The substrate specificity of the *Sorghum* enzyme is limited to aromatic and heteroaromatic aldehydes. The use of (*S*)-oxynitrilase from *Hevea brasiliensis* and a crude recombinant protein which is prepared by overexpression in *Pichia pastoris*, in particular, considerably enhances the scope of substrates for (*S*)-oxynitrilases.[14–16] Acetone cyanohydrin lyase [EC 4.1.2.37] from the leaves of *Manihot esculenta*, previously overexpressed in *E. coli*, further extends the substrate range.[17] The above-mentioned recombinant techniques have made (*S*)-oxynitrilase easily available in large quantities for those who have the above enzyme preparations at hand.

The substrate specificity of the almond enzyme is relatively wide including aliphatic and aromatic aldehydes as well as aliphatic ketones as substrates.[7–9,13] Our previous work for the almond meal-catalysed synthesis of mono- and di-substituted (*R*)-mandelonitriles revealed, however, that bulky substituents in the substrate hamper the reaction.[13] The aim of this work was to find novel sources of (*R*)-oxynitrilase which are capable of transforming sterically hindered arene- and alkylcarbaldehydes to the corresponding (*R*)-cyanohydrins. For that purpose, whole cell preparations (called meal) from mature apple seeds and cherry, apricot and plum pits as the novel sources of (*R*)-oxynitrilase were tested for the condensation of hydrogen cyanide with aldehydes **1a–i** and **3a–f** (Schemes 1 and 2) in aqueous diisopropyl ether.

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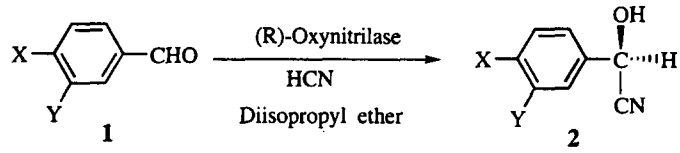
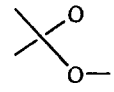
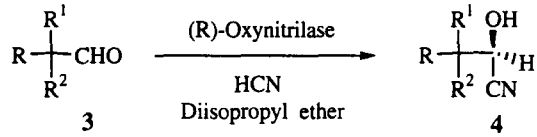


a X = Bu^tCO_2, Y = H
b X = AcO, Y = H
c X = H, Y = H
d X = PhO, Y = H
e X = Pr^i, Y = H
f X = HO, Y = H
g X = MeO, Y = MeO
h X = AcO, Y= $AcOCH_2$
i



Scheme 1.



a R = Et, $R^1 = R^2 = H$
b R = *n*-Butyl, $R^1 = R^2 = H$
c R = *n*-Octyl, $R^1 = R^2 = H$
d $R = R^1 = R^2 = Me$
e R = Et, R^1 = Me, $R^2 = H$
f R = *n*-Butyl, R^1 = Et, $R^2 = H$

Scheme 2.

Results and discussion

The condensation of hydrogen cyanide with aldehydes **1c**, **1i** and **3d** was first conducted in the presence of almond, apple, apricot, cherry and plum meal in diisopropyl ether containing 0.1 M tartrate buffer [2% (v/v), pH 5.4] at 5°C. Almond meal itself was a commercial product. In the case of the other preparations, the meal obtained from the seeds or kernels of mature garden fruits was defatted by extraction with organic solvents (Experimental section). According to the results of Table 1, all the enzyme preparations effectively lead to the formation of (*R*)-mandelonitrile (**2c**) with high enantiomeric excess (ee close to 100%). In the case of sterically more hindered substrates **1i** and **3d**, however, differences both in the terms of reactivity and enantioselectivity are obvious, apple meal being most promising and plum meal the worst as a catalyst. Thus, the properties of (*R*)-oxynitrilases from different cyanogenic plants clearly differ from each others. This was expected on the basis of species-specific and group-specific differences in some kinetic and other properties of purified cyanogenic plant oxynitrilases previously reported.[18–20]

The almond, apple and cherry preparations were selected as (*R*)-oxynitrilase sources for further studies. Clearly, the almond and cherry meal show highly similar properties in catalysing the formation of the (*R*)-stereogenic centre from the corresponding prochiral carbonyl carbon (Table 2). As can be expected on the basis of the screenings of Table 1, the enantioselectivity of the apple enzyme is often higher than that of the other enzymes. Accordingly, high enantioselectivity is observed especially

Table 1. Synthesis of **2c**, **2i** and **4d** in diisopropyl ether[a] in the presence of various oxynitrilase sources at 5°C by method 2

Product	Source of Enzyme	Time/h	Conversion/%	ee/%
2c	Almond	24	95	99
	Apple	26	96	99
	Apricot	23	81	99
	Cherry	23	97	99
	Plum	23	89	99
2i	Almond	70	3	30
	Apple	67	6	73
	Plum	67	2	8
4d	Almond	24	73	70
	Apple	24	99	90
	Plum	24	42	56

[a]Contains 0.1 M tartrate buffer [2% (v/v), pH 5.4].

for the apple meal-catalysed synthesis of aromatic cyanohydrins which have large substituents in the benzene ring (compounds **2a**, **e**, **h** and **i**).

In the case of the almond or cherry enzymes, the corresponding aldehydes are less reactive or lead to products with moderate enantiopurity. The reaction conditions of this work correspond to those previously used for the almond meal catalysis.[13] In the preparation of an important salbutamol (β-adrenergic drug[21]) precursor **2h**, the validity of the system for the apple case was checked with respect to the amount and pH of the tartrate buffer in diisopropyl ether (Table 3). In the terms of good reactivity and high enantioselectivity, and as in the almond case, only 2% (v/v) of the buffer is needed for the apple enzyme to work. Moreover, at low buffer content pH seems to be unimportant.

In accordance with the previous results,[11] almond meal catalysis allows the preparation of short-chain aliphatic (*R*)-cyanohydrins (compounds **4a** and **b**; Table 2) with high yield and enantiopurity. However, longer reaction times are needed and the enantiopurity of the product gets worse with increasing chain length (compound **4c**). Again apple meal is more favourable than almond meal as a catalyst. The same is true for the formation of tertiary alcohol **4d** which is obtained with high chemical yield and enantiopurity (ee 90%) in the presence of apple meal. Moreover, for racemic aliphatic aldehydes **3e** and **f** as substrates, apple meal is a preferable catalyst in the case of the sterically more hindered α-ethyl substituted aldehyde. Fortunately, the peaks for the four stereoisomers resulting in the chemical condensation of hydrogen cyanide with racemic **3e** and **f** can be separated on the chiral GLC column. Based on the use of highly (*R*)-selective almond and apple meal as catalysts and on the main peaks 1 and 2 (according to the retention times) obtained for the enzymatic condensations, two diastereomeric compounds (2*R*,3*R*) and (2*R*,3*S*), both with high enantiopurity (ee>92%), were formed from each aldehyde **3e** and **f** (two last rows; Table 2). According to this reasoning, peaks 1 and 3 belong to one enantiomer pair, peaks 2 and 4 belonging to the other pair. However, it is not known which one of the peaks 1 and 2 belongs to the former diastereomer and which one to the latter. The diastereomeric excess [de=2% (**4e**; almond and apple meal), de=9% (**4f**; almond meal) and de=15% (**4f**; apple meal)] of these condensations is low. Thus, the almond and apple enzymes do not properly recognise the stereogenic centre at the α-position of the aldehyde although the prochiral aldehyde carbon is transformed to the (*R*)-stereogenic centre with high stereoselection. In accordance with this, also the enantiopurity of the unreacted aldehyde is negligible. Thus, *e.g.*, in the apple meal-catalysed reaction at 70% conversion ee=8% is obtained for the unreacted **3f**. Apple meal catalysis was used for

Table 2. Synthesis of **2a–i** and **4a–f** in diisopropyl ether[a] in the presence of various oxynitrilase sources at 5°C by method 2

Product	Almond meal			Apple meal			Cherry meal		
	Time/h	Conversion/%	ee/%	Time/h	Conversion/%	ee/%	Time/h	Conversion/%	ee/%
2a	70	3	30	63	12	82	72	3	2
2b	64	85	96[b]	-	-	-	-	-	-
2c	24	95	99	26	96	99	23	97	99
2d	480	90	95[b]	216	92	99	-	-	-
2e	336	43	86	336	49	94	336	39	90
2f	68	64	96[b]	68	66	89	71	57	89
2g	66	82	93[b]	68	86	93	71	84	94
2h	69	30	15	48	58	82	-	-	-
2i	70	3	30	67	6	73	-	-	-
4a	24	95	97	24	98	99	24	96	98
4b	42	72	97	42	71	98	42	83	97
4c	120	33	68	120	70	92	-	-	-
4d	24	73	70	24	99	90	-	-	-
4e	24	95	98[c]/92[d]	24	98	98[c]/94[d]	-	-	-
4f	48	9	94[c]/96[d]	72	70	98[c]/98[d]	-	-	-

[a]Contains 0.1 M tartrate buffer [2% (v/v), pH 5.4]. [b]Ref. 13. [c]Chiral GLC; peaks 1 and 3 taken as peaks for the enantiomers. [d]Chiral GLC; peaks 2 and 4 taken as peaks for the enantiomers.

the preparative-scale synthesis of the cyanohydrins **2** and **4** (**4e** and **f** as the diastereomeric mixture of two stereoisomers) described in the Experimental section.

Two methods which differ in the ways hydrogen cyanide is introduced into the reaction mixture was previously used for the biocatalytic transformations of prochiral arenecarbaldehydes to the corresponding (*R*)-cyanohydrins: *method 1* where acetone cyanohydrin (ACH) serves as the source of hydrogen cyanide *in situ* in the reaction vessel and *method 2* which exploits hydrogen cyanide evaporating from diisopropyl ether and diffusing into the reaction mixture in a two compartment reaction vessel.[12,13] As a benefit of the latter method, neat hydrogen cyanide at an appropriate concentration is transported all the time into the reaction mixture. This method has been used also

Table 3. The effect of the amount and pH of the tartrate buffer (0.1 M) in diisopropyl ether for the apple meal-catalysed synthesis of cyanohydrin **2h** at 5°C by method 2

Buffer/% (v/v)	pH	Time/h	Conversion/%	ee/%
100	3.75	48	91	33
5	5.40	48	55	76
5	3.75	48	50	82
2	5.40	48	58	82
2	3.75	48	55	86
0.5	5.40	72	14	18
0.5	3.75	72	10	20

Table 4. Diffusion of HCN from diisopropyl ether (DIPE), hexane or water in compartment B (V=10 ml) into the reaction mixture[a] in A in a two compartment reaction vessel at 5°C within 6 h

System in B	HCN[a]/mmol in B (initial)	ACH/mmol in B (initial)	HCN[b]/mmol in A
HCN-DIPE	10	-	1.6
HCN-DIPE	5	-	1.0
HCN-DIPE2	2	-	0.5
ACH-DIPE-Amberlite	-	11	<0.1
ACH-DIPE-Amberlite	-	11[b]	0.2
ACH-Hexane-Amberlite	-	11[b]	0.5
ACH-Water-NaOH (0.25 M)	-	11[b]	0.6

[a]DIPE (16.4 ml) containing toluene (165 µl) as a internal standard. [b]At 25 °C.

throughout this work. In order to avoid the handling of hydrogen cyanide in ether, a modified method (*method 3*) connecting the benefits of the two earlier methods was studied. In this method, the base-catalysed decomposition of acetone cyanohydrin first provides hydrogen cyanide *in situ* in one compartment B and the product then evaporates and diffuses into the reaction mixture in another compartment A of the two compartment reaction vessel. For oxynitrilase-catalysed synthesis, the concentration of hydrogen cyanide in the reaction mixture must always be sufficiently high. Accordingly, the decomposition of acetone cyanohydrin and its evaporation thereafter must be fast processes. Clearly, acetone cyanohydrin (1.1 M) dissolved in water in the presence of sodium hydroxide (kept at 25°C under stirring) fulfils this demand as can be seen comparing the results of Table 4 (rows 3 and 7). Condensation and polymerisation reactions of hydrogen cyanide to coloured products take place in the presence of water, however, leading us to reject the economical aqueous system.[22] Acetone cyanohydrin dissolved in hexane in the presence of a basic Amberlite IRA-904 resin finally served as the source of hydrogen cyanide in method 3.

Methods 2 and 3 were used for the almond, apple and cherry meal-catalysed syntheses of some (*R*)-cyanohydrins (Table 5). According to the results it is possible to replace hydrogen cyanide of method 2 by acetone cyanohydrin used in method 3. Enantioselectivity is usually independent of a method. In the case of method 3, however, reactivity is somewhat lower compared to method 2 evidently because of the lower amount of hydrogen cyanide (within 6 hours 0.5 and 1.6 mmol, respectively; Table 4) introduced into compartment A. On the other hand, reactivity for the almond meal-catalysed formation of mandelonitrile **2c** is not affected over the same concentration range when method 2 is used (rows

Table 5. Synthesis of (*R*)-cyanohydrins in diisopropyl ether[a] in the presence of various oxynitrilase sources at 5°C by methods 1 [ACH (1.1 M)], 2 [HCN (1.0 M) in diisopropyl ether] and 3 [ACH (1.1 M) in hexane]

Product	Source of Enzyme	Time/h	Conversion/%	ee/%	Method
2c	Almond	24	92	87	1
		24	95	99	2
		23	98	99	2[b]
		23	92	99	2[c]
		24	92	98	3
2f	Apple	68	66	89	2
		72	42	79	3
2f	Cherry	71	57	89	2
		71	46	88	3
2g	Apple	68	86	93	2
		70	75	90	3
2g	Cherry	71	84	94	2
		48	67	96	3

[a]Contains 0.1 M tartrate buffer [2% (v/v), pH 5.4]. [b]0.5 M HCN in diisopropyl ether in compartment B. [c]0.2 M HCN in compartment B.

2–4; Table 5). The temperature difference between the two compartments of the vessel (25°C in B and 5°C in A) may also affect reactivity in method 3.

Conclusions

Almond meal as a commercial and economical (*R*)-oxynitrilase source effectively transforms various types of aldehydes to the corresponding (*R*)-cyanohydrins. The present results demonstrate the possibility of enhancing the scope of substrates by introducing novel sources (apple, apricot, cherry and plum meal) of (*R*)-oxynitrilase for the condensation of hydrogen cyanide with prochiral aldehydes. In this respect, apple meal is by far the most favourable as a catalyst allowing the highly enantioselective syntheses of important sterically restricted or long-chain aliphatic and aromatic cyanohydrins. The present results also demonstrate that the almond and apple enzymes do not properly recognise other stereogenic centres except the forming (*R*)-centre in the molecule. A two compartment reaction vessel with etheral hydrogen cyanide in compartment B and the reaction mixture in another A is conveniently used for enzymatic cyanohydrin formation.[13] It is shown now that acetone cyanohydrin (method 3) can replace hydrogen cyanide in compartment B as the source of hydrogen cyanide.

Experimental

Enzyme preparations and chemicals

Defatted almond meal was purchased from Sigma. The seeds of typical Finnish garden apples were collected and frozen (−20°C). The pits of apricot, cherry and plum were crushed and the kernels were collected and also frozen. After grinding in a mortar the preparation was washed first with ethyl acetate until the filtrate was uncoloured and finally with acetone. After filtration with suction the preparation was air-dried. Defatted meal was stored at 4°C.

Diisopropyl ether was distilled before use. Amberlite^{R} IRA-904 ion-exchange resin was purchased from Aldrich. Commercial aldehydes were obtained from Aldrich or Merck and were distilled or recrystallized before use. 4-Hydroxy-3-hydroxymethylbenzaldehyde was a gift from Leiras Oy.

Aldehydes **1a**, **b**, **h** and **i** were prepared by protecting the free hydroxyl groups in the corresponding substituted benzaldehydes with acetic anhydride, pivaloyl chloride or acetone. Acetone cyanohydrin (a product of Aldrich) was distilled before use. Neat hydrogen cyanide was prepared by adding potassium cyanide solution dropwise into dilute sulphuric acid[13] and leading the hydrogen cyanide formed into dry diisopropyl ether. The etheral solution was stored in a freezer. Racemic cyanohydrins for retention time determinations in GLC were prepared from the corresponding aldehydes and hydrogen cyanide using a known method.[12,13] Hydrogen cyanide content was determined by GLC using toluene as an internal standard.

Methods and syntheses

^{1}H (TMS as an internal standard) and ^{13}C NMR spectra were measured on a Lambda GX 400 spectrometer. Two-dimensional (2D) ^{1}H NMR spectra used in analysing the peaks of compounds **4e** and **4f**, were also obtained on the same instrument. MS spectra were recorded on a VG Analytical 7070E instrument equipped with a VAXstation 3100 M76 system. The enantiomeric purities of the cyanohydrins were determined after acylation with the chiral GLC method using Chrompack CP-Cyclodextrin-β-2,3,6-M-9 column.[12,13] Optical rotations were measured using a JASCO DIP-360 polarimeter.

Method 1

In a typical experiment, the enzyme preparation (74 mg) was mixed with diisopropyl ether (6.5 ml). Tartrate buffer (0.6 ml, 0.1 M, pH 5.4) and a freshly distilled aldehyde (0.5 mmol) were added. The mixture was stirred half an hour at room temperature and acetone cyanohydrin (5.5 mmol) was added. The reactions were followed by taking samples at intervals and using the chiral GLC method.[13]

Method 2

In a typical experiment, the enzyme preparation (150 mg) was mixed with diisopropyl ether (16 ml) in the compartment A of the two-compartment reaction vessel. Tartrate buffer (330 µl, 0.1 M, pH 5.4) and a freshly distilled aldehyde (1.0 mmol) were added. The reaction mixture was stirred at 5°C. The other compartment B contained diisopropyl ether (10 ml) and hydrogen cyanide (10 mmol) also at 5°C. The reaction was followed and the product was handled as shown in method 1.[13]

Method 3

In the compartment A of the two-compartment reaction vessel, the enzyme preparation (150 mg) was mixed with diisopropyl ether (16 ml). Tartrate buffer (330 µl, 0.1 M, pH 5.4) and a freshly distilled aldehyde (1.0 mmol) were added. The reaction mixture was stirred at 5°C. In compartment B, diisopropyl ether (10 ml) containing acetone cyanohydrin (11 mmol) and Amberlite^R IRA-904 ion-exchange resin (500 mg) were stirred at 25°C. The reaction was followed and the product was handled as shown in method 1.

The reactions for the formation of cyanohydrins **2** and **4** in the presence of apple meal were stopped by filtering off the enzyme preparation. For the reactions which proceeded to more than 90%, the unreacted aldehyde was removed by washing 5 times with 10% $NaHSO_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. In the case of lower conversions, the aldehyde was not washed out but the solvent was directly evaporated. The isolated yield was calculated on the basis of conversions. The spectroscopic data were obtained from chemically prepared racemic cyanohydrins.

(R)-2-Hydroxy-2-(4-trimethylacetyloxyphenyl)acetonitrile $C_{13}H_{15}NO_3$ (2a)

The product corresponding to 12% conversion; $[\alpha]_D^{25}$ =+18.7 ($CHCl_3$, c=6.7). The product was acetylated before the spectroscopic analysis. ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 9H, *tert*-C_4H_9); 2.16 (s, 3H, CH_3); 6.42 (s, 1H, CH); 7.14–7.17 (m, 2H, H_{arom}); 7.53–7.56 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$); δ

20.48 (CH_3); 27.07 (CH_3); 39.17 (C); 62.67 (CH); 115.99 (CN); 122.49, 129.03 129.28 and 152.63 (C_{arom}), 168.87 and 176.72 (CO).

(R)-2-Hydroxy-2-phenylacetonitrile C_8H_7NO (2c)

Yield 90%; $[\alpha]_D^{25}$=+40.0 ($CHCl_3$, c=4.5). ^1H NMR ($CDCl_3$): δ 3.49 (s, 1H, OH); 5.57 (s, 1H, CH); 7.43–7.53 (m, 5H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 63.84 (CH); 118.82 (CN); 126.64, 129.48 129.82, 130.15, 130.21 and 135.27 (C_{arom}).

(R)-2-Hydroxy-2-(4-phenoxyphenyl)acetonitrile $C_{14}H_{11}NO_2$ (2d)

Yield 90%; $[\alpha]_D^{25}$=+21.9 ($CHCl_3$, c=6.9). ^1H NMR ($CDCl_3$): δ 3.71 (s, 1H, CH); 7.26–7.17–7.68 (m, 9H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 63.12 (CH); 117.56 (CN); 118.80, 119.53, 120.47, 124.10, 125.02, 128.45, 129.71, 129.97, 130.17, 132.13, 156.21 and 158.90 (C_{arom}).

(R)-2-Hydroxy-2-(4-isopropylphenyl)acetonitrile $C_{11}H_{13}NO$ (2e)

The product corresponding to 49% conversion; $[\alpha]_D^{25}$=+21.8 (EtOAc, c=4.3). ^1H NMR ($CDCl_3$): δ 1.24 (d, 3H, CH_3), 1.26 (d, 3H, CH_3); 2.95 (m, 1H,CH); 3.06 (s, 1H, OH); 5.49 (s, 1H, CH); 7.29 (d, 2H, H_{arom}), 7.45 (d, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 23.81 (CH_3), 23.89 (CH_3); 33.93 (CH); 63.54 (CH); 118.92 (CN); 126.83, 127.32, 132.69 and 150.96 (C_{arom}).

(R)-2-Hydroxy-2-(4-hydroxyphenyl)acetonitrile $C_8H_7NO_2$ (2f)

The product corresponding to 66% conversion; $[\alpha]_D^{25}$ =+30.4 (EtOAc, c=4.9). The product was acetylated before the spectroscopic analysis. ^1H NMR ($CDCl_3$): δ 2.17 (s, 3H, CH_3); 2.33 (s, 3H, CH_3); 5.48 (s, 1H, CH); 7.19 (d, 2H, H_{arom}), 7.56 (d, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 20.46 (CH_3); 21.10 (CH_3); 62.26 (CH); 115.94 (CN); 122.56, 129.33 and 152.14 (C_{arom}), 168.83 and 169.02 (CO).

(R)-2-Hydroxy-2-(3,4-dimethoxyphenyl)acetonitrile $C_{10}H_{11}NO_3$ (2g)

The product corresponding to 86% conversion; $[\alpha]_D^{25}$=+24.4 (EtOAc, c=4.8).^1H NMR ($CDCl_3$): δ 3.10 (s, 1H, OH); 3.90 (s, 6H, CH_3); 6.89 (d, 1H, H_{arom}); 7.02 (d, 1H, H_{arom}); 7.08 (d, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 56.02 (CH_3); 56.22 (CH_3); 63.66 (CH); 109.65 and 111.14 (C_{arom}); 118.89 (CN); 119.67, 128.15, 149.50 and 150.22 (C_{arom}).

(R)-2-Hydroxy-2-(3-acetyloxymethyl-4-acetyloxyphenyl)acetonitrile $C_{13}H_{13}NO_5$ (2h)

The product corresponding to 58% conversion; $[\alpha]_D^{25}$= +15.1 ($CHCl_3$, c=11.3). ^1H NMR ($CDCl_3$): δ 2.10 (s, 3H,CH_3); 2.35 (s, 3H, CH_3); 5.09 (d, 1H, CH_2); 5.45 (d, 1H, CH); 7.10–7.20 (d, 1H, H_{arom}); 7.45–7.55 (q, 1H, H_{arom}); 7.55–7.65 (d, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 20.81 (CH_3); 20.89 (CH_3); 61.08 (CH_2); 62.93 (CH); 118.69 (CN); 123.62, 127.92, 129.78, 130.84, 133.25 and 156.82 (C_{arom}), 169.12 and 170.68 (CO). MS: m/z 263 (M, 1), 236 (3), 221 (1), 194 (52), 161 (20), 152 (10), 134 (70), 106 (22), 78 (12), 51 (10), 43 (100).

(R)-2-Hydroxy-2-(O,O-isopropylidene-4-hydroxy-3-hydroxymethylphenyl)acetonitrile $C_{12}H_{13}NO_3$ (2i)

The product corresponding to 6% conversion; $[\alpha]_D^{25}$= +13.0 ($CHCl_3$, c=0.75). ^1H NMR ($CDCl_3$): δ 1.53 (s, 3H, CH_3); 1.54 (s, 3H, CH_3); 4.75–4.84 (q, 2H, CH_2); 5.42–5.43 (d, 1H, CH); 7.13–7.14 (m, 1H, H_{arom}); 7.27–7.28 (m, 1H, H_{arom}); 7.29–7.30 (m, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 24.59 (CH_3); 24.74 (CH_3); 60.66 (CH_2); 63.25 (CH); 100.14 (C); 117.91 (C_{arom}), 118.96 (CN); 120.01, 123.46, 126.92, 127.35 and 152.42 (C_{arom}). **MS**: m/z 219 (M, 35), 192 (27), 175 (10), 161 (100), 150 (17), 143 (17), 134 (78), 119 (45), 106 (67), 89 (10), 78 (55), 51 (37), 43 (40), 39 (25).

(R)-2-Hydroxypentanenitrile C_5H_9NO (4a)

Yield 92%; $[\alpha]_D^{25}$= +23.4 ($CHCl_3$, c=4.1). ^1H NMR ($CDCl_3$): δ 0.99 (t, 3H, CH_3); 1.50–1.59 (m, 2H, CH_2); 1.76–1.88 (m, 2H, CH_2); 2.7–3.1 (br, s, 1H, OH); 4.49 (t, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 13.40 (CH_3); 17.90 and 37.14 (CH_2); 61.10 (CH); 120.07 (CN).

(R)-2-Hydroxyheptanenitrile $C_7H_{13}NO$ (4b)

The product corresponding to 71% conversion; $[\alpha]_D^{25}$= +12.5 ($CHCl_3$, c=6.0).^{1}H NMR ($CDCl_3$): δ 0.91 (tt, 3H, CH_3); 1.24–1.38 (m, 4H, 2*CH_2); 1.47–1.53 (m, 2H, CH_2); 1.82–1.88 (m, 2H, CH_2); 2–6–2.8 (br, s, 1H, OH); 4.48 (t, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 13.90 (CH_3); 22.41, 24.21, 31.08 and 35.23 (CH_2); 61.41 (CH); 119.99 (CN).

(R)-2-Hydroxyundecanenitrile $C_{11}H_{21}NO$ (4c)

The product corresponding to 70% conversion; $[\alpha]_D^{25}$= +10.9 ($CHCl_3$, c=5.9). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, CH_3); 1.20–1.40 (m, 12H, 6*CH_2); 1.45–1.55 (m, 2H, CH_2); 1.82–1.87 (m, 2H, CH_2); 2–6–2.8 (br, s, 1H, OH); 4.47 (t, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 14.12 (CH_3); 22.69, 24.55, 28.50, 28.94, 29.15, 29.37, 31.87 and 35.27 (CH_2); 61.41 (CH); 120.02 (CN).

(R)-2-Hydroxy-3,3-dimethylbutanenitrile $C_6H_{11}NO$ (4d)

Yield 90%; $[\alpha]_D^{25}$= +17.4 ($CHCl_3$, c=5.5). ^{1}H NMR ($CDCl_3$): δ 1.09 (s, 9H, *tert*-C_4H_9); 2.60–2.90 (s, 1H, OH); 4.13 (s, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 24.97 (3*CH_3); 35.50 (C); 70.84 (CH); 119.10 (CN).

(2R,3R) and (2R,3S)-2-Hydroxy-3-methylpentanenitrile $C_6H_{11}NO$ (4e)

Yield 90%. ^{1}H NMR ($CDCl_3$): δ 0.97 and 0.96 (t, 3H, CH_3); 1.09 (t, 3H, CH_3); 1.20–1.70 (m, 2H, CH_2); 1.70–1.90 (m, 1H, CH_2); 2.60–2.70 (br, s, 1H, OH); 4.38 (t, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 11.14 and 11.30 (CH_3); 13.40 (CH_3); 24.46 and 24.91 (CH_2); 39.42 and 39.48 (CH); 65.63 and 65.95 (CH); 119.26 and 119.64 (CN). **MS**: m/z 114 (M+1, 1), 86 (14), 57 (100).

(2R,3R) and (2R,3S)-2-Hydroxy-3-ethylheptanenitrile $C_9H_{17}NO$ (4f)

The product corresponding to 70% conversion. ^{1}H NMR ($CDCl_3$): δ 0.90–0.92 (over, m, 3H, CH_3); 0.94–0.99 (over, m, 3H, CH_3); 1.30–1.33 (m, 6H, CH_2); 1.34–1.69 (m, 3H, CH and CH_2); 2.4–2,6 (br, s, 1H, OH) 4.50–4.52 (q, 1H, H); ^{13}C NMR ($CDCl_3$): δ 11.09 and 11.26 (CH_3); 13.94 (CH_3); 22.02 and 22.16 (CH_2); 22.82 (CH_2); 28.54 and 28.70 (CH_2); 28.95 and 29.03 (CH_2); 44.10 (CH); 64.14 and 64.26 (CH); 119.49 (CN). **MS**: m/z 155 (M, 0.1), 140 (0.1), 99 (16), 82 (8), 72 (22), 58 (18), 57 (71), 55 (25), 43 (100), 41 (84), 39 (22).

Acknowledgements

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