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Approach to (*R*)- and (*S*)-ketone cyanohydrins using almond and apple meal as the source of (*R*)-oxynitrilase

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Abstract: The synthesis of aliphatic and aromatic (*R*)-ketone cyanohydrins through the addition of hydrogen cyanide to the corresponding ketones and the synthesis of the (*S*)-enantiomers through the kinetic resolution of racemic ketone cyanohydrins has been studied in the presence of almond or apple meal. Substrate tolerance of the (*R*)-oxynitrilases towards ketones ($R^1R^2C{=}O$) is highly restricted compared to that of structurally similar aldehydes, reactivity following the order of H>Me≫Et for R^2. In the case of aromatic methyl ketones reactivity difference ($C_6H_5 \gg p$-Me-C_6H_4 for R^1) is notable. © 1997 Elsevier Science Ltd

The synthesis of optically active aldehyde cyanohydrins by oxynitrilase-catalysed addition of hydrogen cyanide to prochiral aldehydes has been the goal of extensive and successful studies during recent years.[1–16] The synthesis of optically active ketone cyanohydrins, on the other hand, has been less studied. (*R*)-Oxynitrilases [E.C. 4.1.2.10] isolated from almonds (*Prunus amygdalus*) and flax (*Linum usitatissimum*) were found to catalyse the enantioselective addition of hydrogen cyanide to methyl or ethyl alkyl ketones, yielding the corresponding (*R*)-ketone cyanohydrins.[16–18] In the case of the flax enzyme, however, enantiopurities and absolute configurations remain mostly unresolved.[16] The almond enzyme was also useful for the preparation of some aliphatic (*S*)-ketone cyanohydrins through kinetic resolution.[19] (*S*)-Oxynitrilase [E.C. 4.1.2.37] isolated from *Manihot esculenta* and overexpressed in *E. coli* shows high potential (ee of the order of 90% or lower) for the preparation of aliphatic and even aromatic (*S*)-ketone cyanohydrins.[13] Unfortunately, the enzyme preparation is not commercially available. On the other hand, the commercial (*S*)-oxynitrilase [E.C. 4.1.2.11] from *Sorghum bicolor* does not accept ketones as substrates.[7]

Relaying on the (*R*)-selective almond enzyme, we have now exploited almond meal for the preparation of (*R*)-ketone cyanohydrins **2a–h** through the addition of hydrogen cyanide with the corresponding prochiral ketone (Scheme 1A) and (*S*)-ketone cyanohydrins **2a–i** through the kinetic resolution of the corresponding racemic cyanohydrin with or without the presence of aldehyde **3** (Scheme 1B). As previously shown, apple meal (another rich source of (*R*)-oxynitrilase) is more favourable than almond meal for sterically hindered aldehydes (*e.g.*, pivalaldehyde) as substartes.[8] Accordingly, some apple meal-catalysed additions were also studied in this work.

Enzymatic kinetic resolution in the presence of hydrolytic enzymes is the most commonly used biocatalytic way to optically active alcohols.[20] The method was previously carried out in the case of aldehyde cyanohydrins by lipases.[21,22] Unfortunately, lipases rarely accept tertiary alcohols as substrates.[20] To our knowledge, the microbial hydrolysis of ketone cyanohydrin acetates on incubation with grown cells of *Pichia miso* IAM 4682 is the only method reported for the preparation of the less reactive (*S*)-ketone cyanohydrin acetates.[23] This further underlines the importance of oxynitrilase methods for the preparation of the enantiomers of ketone cyanohydrins.

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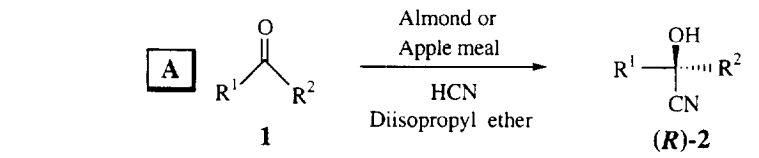
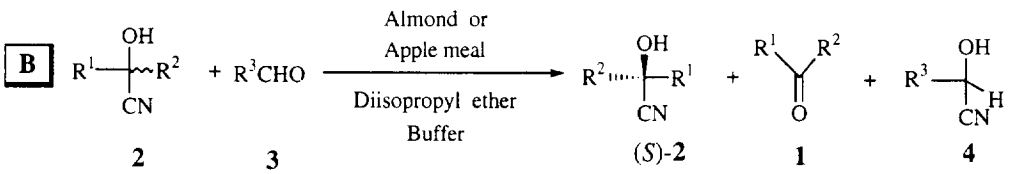


1 (*R*)-2

a R^1=Bu, R^2 = Me **d** R^1 = n-Hexyl, R^2 = Et **g** R^1 = Ph, R^2 = Et
b R^1 = ^{t}Bu, R^2 = Me **e** R^1 = Ph, R^2 = Me **h** R^1 = Ph, R^2 = $CHCl_2$
c R^1 = n-Pentyl, R^2 = Me **f** R^1 = *p*-Me-Ph, R^2 = Me **i** R^1 = ^{i}Pentyl, R^2 = Me



2 3 (*S*)-2 1 4

Scheme 1.

Results and discussion

The synthesis of (*R*)-cyanohydrins **2a–h** in the presence of almond or apple meal was studied in diisopropyl ether containing tartrate buffer [2% (v/v), 0.1 M, pH 5.4] at 5°C (Scheme 1A, Table 1). In the synthesis, the corresponding ketone reacts with hydrogen cyanide which freely evaporates from a solution in diisopropyl ether in one compartment of the reaction vessel and ends up to the other where it dissolves into the reaction mixture (previously called method 2).[7,8] Under such conditions, chemical condensation to racemic cyanohydrins is effectively suppressed except in the case of dichloromethyl ketone **1h** where the electron deficiency of the carbonyl carbon favours nonenzymatic condensation. Aliphatic methyl ketones **1a–c** are transformed to the corresponding (*R*)-cyanohydrins with high enantiopurity (ee 96–99%). Compared to the almond meal-catalysed synthesis of sterically similar aldehyde cyanohydrins reactivity now is considerably lower. Thus, the almond meal-catalysed synthesis of (*R*)-2-hydroxyheptanenitrile from hexanal (corresponding to **1c** with R^2=H) proceeds to 72% conversion within 42 hours (ee 97%) compared to 18 days needed for 76% conversion in the case of methyl ketone **1c** (R^2=Me) as a substrate (Table 1; row 5).[8] Fortunately, reactivity can be considerably increased by increasing the enzyme content (Table 1; row 2 compared to row 1). Reactivity turns even worse when aromatic methyl ketones **1e** and **f** serve as substrates although enantioselectivity still remains considerably high. Moreover, the methyl substituent in the benzene ring significantly affects reactivity. As a comparison, the corresponding aldehydes **1e** and **f** (R^2=H) under the otherwise same conditions have practically reacted within 1 and 2 days, respectively.[7] Ethyl ketones **1d** and **g** are almost unreactive under the reaction conditions, indicating that the ability of a carbonyl compound to act as a substrate for the almond enzyme decreases with increasing size of the group R^2. Clearly, apple meal as a catalyst does not improve reactivity and/or enantioselectivity for the synthesis of ketone cyanohydrins. This is against the previous results for the formation of aldehyde cyanohydrins where apple meal was found to accept even sterically hindered aldehydes.[8]

In synthetic chemistry, the oxynitrilase-catalysed formation of optically active cyanohydrins is a general goal.[1–3] In nature, however, oxynitrilases typically catalyse the decomposition of cyanohydrins, mandelonitrile (Scheme 1B, **4**; R^3=C_6H_5) being the natural substrate of the almond enzyme. The ability of the almond enzyme to catalyse the decomposition of acetone cyanohydrin is also clearly demonstrated.[5–8,15] In the present work, the reversible nature of oxynitrilase catalysis has been studied for the kinetic resolution of racemic cyanohydrins **2a** and **e** in the presence of almond meal in

Table 1. Synthesis of (*R*)-**2a–h** in diisopropyl ether[a] in the presence of almond or apple meal (150 mg/mmol substrate) at 5°C

Substrate	Source of enzyme	Time/h	Conversion/%	ee/%
1a	Almond	168	60	96
1a	Almond[b]	24	73	99
1b	Almond	192	45	97
1b	Apple	192	40	99
1c	Almond	432	76	98
1d	Almond	336	2	76
1e	Almond	120	5	95
1e	Almond	312	14	90
1e	Apple	168	13	93
1f	Almond	504	2	80
1g	Almond	144	<1	20
1g	Apple	96	1	20
1h	Almond	144	67	0
1h	Apple	96	69	5

[a]Contains tartrate buffer [2% (v/v), 0.1 M, pH 5.4]. [b]500 mg/mmol substrate.

diisopropyl ether containing tartrate buffer [7% (v/v), 0.1 M, pH 5.4] at 25°C (Scheme 1B in the absence of **3**). The (*R*)-enantiomer then selectively decomposes to ketones **1a** and **e** leaving the (*S*)-enantiomer of **2** unreacted. Unfortunately, the ee values of 37% obtained for (*S*)-**2a** and 88% obtained for (*S*)-**2e** (conversions 53 and 57%, respectively) are disappointingly low. As an explanation, the ketone **1** enzymatically reacts back to the (*R*)-cyanohydrin **2**, making the preparation of the corresponding highly enantiopure (*S*)-cyanohydrin impossible. Thus, *e.g.*, for the resolution of **2a** the drop of ee is from 46 to 37% at the conversion range from 35 to 53% although the enantiopurity of the less reactive (*S*)-counterpart is expected to increase with conversion. Clearly, there is a need to trap the hydrogen cyanide delivered during the resolution process.

For decades, synthetic chemists have exploited an interchange between a ketone cyanohydrin and an aldehyde for the preparation of lower water-soluble cyanohydrins in the presence of a base.[24] Under the conditions where the chemical interchange is negligible compared to the corresponding almond (or apple) meal-catalysed reaction, the hydrogen cyanide delivered by the reactive (*R*)-enantiomer of **2** is accepted by aldehyde **3** (Scheme 1B). As a further demand, the formed aldehyde cyanohydrin **4** must be stable enough under the conditions where the (*R*)-cyanohydrin **2** is turned to ketone **1**, leaving (*S*)-**2** unreacted. For that purpose, various aldehydes as hydrogen cyanide acceptors were studied for the almond meal-catalysed resolution of racemic **2a** in diisopropyl ether in the presence of tartrate buffer [7% (v/v), 0.1 M, pH 5.4] at 25°C (Table 2). Compared to lower aliphatic aldehydes **3** (R^3 from Me to *n*-pentyl) benzaldehyde is not a good acceptor as can be seen from a low ee (43%) for the less reactive (*S*)-**2a**. In the presence of almond meal, benzaldehyde yields (*R*)-mandelonitrile (the natural substrate for the enzyme) which in the reversible enzymatic reaction produces hydrogen cyanide as another decomposition product.[25] Thus, hydrogen cyanide is not totally bound to benzaldehyde. As a result, there is a complicated equilibrium between the various components of the reaction mixture, leading to the racemization of the enantiomerically enriched (*S*)-**2a** with time. The unexpectedly low enantiopurity of (*R*)-**4** (Table 2), on the other hand, can be explained by a chemical interchange between ketone and aldehyde cyanohydrins (Table 3). Acetaldehyde or propionaldehyde (**3**; R^3 Me or Et) seem

Table 2. The almond meal-catalysed resolution of racemic **2a** in the presence of aldehyde **3** in diisopropyl ether[a] at 25°C

Aldehyde R^3	Time/h	Conversion/%	(*S*)-**2a** ee/%	(*R*)-**4** ee/%
Ph	312	51	43	-
Pent	144	53	96	92
Pr	165	51	96	85
Et	165	50	99	44
Me	165	50	97	-

[a]Contains 0.1 M tartrate buffer [7% (v/v), pH 5.4].

Table 3. The chemical interchange of racemic **2a**, **e** and **f** with acetaldehyde in diisopropyl ether[a] at 25°C

Table 3. The chemical interchange of racemic **2a**, **e** and **f** with acetaldehyde in diisopropyl ether[a] at 25 °C.

Substrate	Time/h	Conversion/%
2a	144	3
2e	144	23
2f	144	9

[a]Contains tartrate buffer [7% (v/v), 0.1 M, pH 5.4].

to be the most advantageous acceptors of hydrogen cyanide because the obtained cyanohydrins **4** then can be extracted into water or distilled at low temperature.

According to the results of Table 4 for the kinetic resolution of racemic cyanohydrins **2a–i**, lower aliphatic methyl ketones **2a**, **b** and **i** and acetophenone **2e** can be resolved by almond (or apple) meal with short-chain aliphatic aldehydes as hydrogen cyanide acceptors. The method indicates the importance of trapping the hydrogen cyanide with an aldehyde. Thus, the enantiomeric excesses of **2a** and **e** can be increased from *ca.* 40 to >95% and from 88 to 98%, respectively, using acetaldehyde. Branching in R^1 somewhat enhances reactivity (Table 4; rows 1 and 3 or 4 and 13). Ethyl ketones **2d** and **g** as well as dichloromethyl ketone **2h** lead to racemic products, indicating again that substrates with R^2>Me are poorly accepted by (*R*)-oxynitrilase. In accordance with the data in Table 1, there is practically no effect on enantioselectivity when almond meal as a catalyst is replaced by apple meal.

As a conclusion, the enantiomers of ketone cyanohydrins can be prepared using aliphatic and aromatic methyl ketones as substrates for almond and apple meal in diisopropyl ether in the presence of minor amounts of tartrate buffer (0.1 M, pH=5.4). The (*R*)-enantiomers are obtained introducing gaseous hydrogen cyanide into the reaction mixture (method 2 in the connection of aldehyde cyanohydrins[7,8]). The method to the (*S*)-enantiomers is based on the enzymatic interchange between the (*R*)-enantiomer of the racemic ketone cyanohydrin and an aldehyde (corresponds to method 1 in the case of aldehyde cyanohydrins[5–7]). In this method, the (*S*)-enantiomer is properly enriched only if the enzymatic decomposition of another interchange product, (*R*)-aldehyde cyanohydrin, can be suppressed under the reaction conditions. Substrate tolerance of the oxynitrilase enzymes is considerably restricted compared to that observed in the case of sterically similar aldehyde cyanohydrins.

Experimental

Chemicals

Defatted almond meal was purchased from Sigma. Apple meal was prepared from apple seeds as previously described.[8] Diisopropyl ether and commercial aldehydes and ketones were obtained from

Table 4. The almond or apple meal-catalysed resolution of racemic **2a–i** in the presence of aldehyde **3** in diisopropyl ether[a] at 25°C

Substrate	Aldehyde R^3	Time/h	Conversion[b]/%	(*S*)-**2** ee/%
2a	Me	165	50	97
2b	Pr	48	50	99
2b	Me	94	49	98
2c	Pr	672	53	67
2d	Me	432	1	2
2e	Me	72	58	98
2f	Et	336	60	59
2f	Me	408	57	39
2f[c]	Me	408	32	73
2g	Me	144	90	1
2h	Et	168	25	0
2h[c]	Me	144	35	5
2i	Pr	432	40	96

[a]Contains tartrate buffer [7% (v/v), 0.1 M, pH 5.4]. [b]Unreacted **2** in the reaction mixture. [c]Using apple meal as a catalyst.

Aldrich or Merck and were distilled before use. Neat hydrogen cyanide was prepared by adding potassium cyanide solution dropwise into dilute sulphuric acid[7,8] and leading the hydrogen cyanide formed into dry diisopropyl ether. The etheral solution was stored in a freezer. Racemic ketone cyanohydrins were prepared from the corresponding ketones *via* a reaction with trimethylsilylcyanide and ZnI_2 followed by desilylation with 3 M HCl.[26]

^1H (TMS as an internal standard) and ^{13}C NMR spectra were measured on a Lambda GX 400 spectrometer in $CDCl_3$. MS spectra were recorded on a VG Analytical 7070E instrument equipped with a VAXstation 3100 M76 system. The enantiomeric excess of the aldehyde and ketone cyanohydrins were determined after acylation with acetic, propionic or hexanoic acid anhydride by the chiral GLC method using Chrompack CP-Cyclodextrin-β-2,3,6-M-9 column.[7,8] Optical rotations were measured using a JASCO DIP-360 polarimeter.

Method

Synthetic method

In a typical experiment, almond meal (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 ketone (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) at 5°C. The reaction was followed by taking samples at intervals and using the chiral GLC method.[7,8] The reactions for the formation of (*R*)-cyanohydrins **2a–c** were stopped by filtring off the enzyme. The solvent was evaporated under reduced pressure. In the other cases the products were not separated from the reaction mixture. The spectroscopic data were obtained from chemically prepared racemic cyanohydrins.

Resolution methods

In a typical experiment, the enzyme preparation (150 mg) was mixed with diisopropyl ether (16 ml) and tartrate buffer (1.2 ml, 0.1 M, pH 5.4). One of the racemic ketone cyanohydrins **2a–i** (1.5 mmol), and in the case of the interchange reactions freshly distilled aldehyde **3** (1.0 mmol) were added. The reactions were followed as described above. In the case of racemic cyanohydrins **2a** and **2e** the interchange reactions were performed in a gram-scale in the presence of acetaldehyde **3** (R^3=Me). The reactions were stopped by filtering off the enzyme preparation. The unreacted aldehyde and the corresponding cyanohydrin **4** (R^3=Me) were washed out. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The isolated yields were calculated taking the conversion to correspond 100% theoretical yield.

*(R)-2-Hydroxy-2-methylhexanenitrile $C_7H_{13}NO$ **2a***

The product corresponding to 82% conversion; $[\alpha]_D^{25}$=+5.1 ($CHCl_3$, c=5.2).

*(S)-2-Hydroxy-2-methylhexanenitrile $C_7H_{13}NO$ **2a***

Yield 45%; $[\alpha]_D^{25}$=−7.4 ($CHCl_3$, c=6.6). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, CH_3); 1.20–1.30 (m, 2H, CH_2); 1.30–1.50 (m, 2H, CH_2); 1.56 (s, 3H, CH_3) 1.75–1.85 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 14.19 and 22.46 (CH_3); 26.35, 27.73 and 41.50 (CH_2); 68.76 (C); 122.01 (CN). MS: m/z 126 (M−1, 0.1), 112 (3), 100 (5), 94 (1), 85 (5), 84 (6), 71 (100), 68 (10), 58 (20), 57 (38), 43 (24) and 41 (12).

*(R)-2-Hydroxy-2,4-dimethylpentanenitrile $C_7H_{13}NO$ **2b***

The product corresponding to 45% conversion; $[\alpha]_D^{25}$=+4.4 ($CHCl_3$, c=3.5). ^{1}H NMR ($CDCl_3$): δ 1.09 (d, 6H, CH_3); 1.65 (s, 3H, CH_3) 1.70 (d, 2H, CH_2); 1.96 (m, 1H, CH); ^{13}C NMR ($CDCl_3$): δ 15.28, 23.63 and 23.68 (CH_3), 28.89 (CH_2), 49.80 (CH), 68.07 (C), 122.01 (CN). MS: m/z 127 (M, 0.1), 108 (2), 100 (5), 94 (9), 85 (6), 82 (5), 71(100), 57 (45), 43 (95), 41 (53) and 39 (24).

*(R)-2-Hydroxy-2-methylheptanenitrile $C_8H_{15}NO$ **2c***

The product corresponding to 76% conversion; $[\alpha]_D^{25}$=+6.0 ($CHCl_3$, c=5.1); $[\alpha]_D^{25}$ (lit[17])=+2.7 ($CHCl_3$, c=1.12). ^{1}H NMR ($CDCl_3$): δ 0.92 (overl., t 3H, CH_3); 1.20–1.55 (m, 6H, CH_2); 1.61 (s, 3H, CH_3); 1.72–1.80 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$): δ 13.92 and 22.42 (CH_3); 23.93, 27.70, 31.45 and 41.73 (CH_2); 68.77 (C); 122.04 (CN). MS: m/z 140 (M−1, 0.2), 126 (0.5), 114 (2), 108 (1), 99 (2), 95 (1), 85 (1), 71 (68), 68 (10), 58 (22), 55 (10), 43 (100) and 41 (20).

*2-Hydroxy-2-ethyl-2-octanenitrile $C_{10}H_{19}NO$ **2d***

^{1}H NMR ($CDCl_3$): δ 0.90 (t 3H, CH_3); 1.11 (t, 3H, CH_3); 1.29–1.40 (m, 6H, CH_2); 1.47–1.55 (m, 2H, CH_2); 1.67–1.88 (m, 4H, CH_2); ^{13}C NMR ($CDCl_3$): δ 8.23 and 14.02 (CH_3); 22.52, 23.89, 29.07, 31.57, 33.26 and 39.71 (CH_2); 72.92 (C); 121.26 (CN). MS: m/z 169 (M, 0.2), 154 (6), 141 (6), 113 (19), 97 (28), 85 (100), 72 (14), 57 (18) and 43 (32).

*(S)-2-Hydroxy-2-methyl-2-phenylacetonitrile C_9H_9NO **2e***

Yield 40%; $[\alpha]_D^{25}$=−1.8 ($CHCl_3$, c=9.8). ^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3H, CH_3); 2.98 (s, 1H, OH); 7.21–7.23 (m, 2H, H_{arom}); 7.45–7.48 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 31.08 (CH_3); 70.76 (C); 121.42 (CN); 2*124.44, 2*129.01, 129.26 and 139.22 (C_{arom}). MS: m/z 147 (M, 35), 132 (100), 120 (12), 105 (40), 77 (40), 54 (12), 51 (20), 43 (16) and 39 (8).

*2-Hydroxy-2-methyl-2-(4′-methylphenyl)acetonitrile $C_{10}H_{11}NO$ **2f***

^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3H, CH_3); 2.37 (s, 3H, CH_3); 3.07 (s, 1H, OH); 7.39–7.46 (m, 2H, H_{arom}); 7.58–7.61 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 21.09 and 30.92 (CH_3); 70.61 (C); 121.59 (CN); 2*121.59, 2*129.60, 137.83 and 139.22 (C_{arom}). MS: m/z 161 (M, 25), 146 (100), 134 (25), 119 (70), 91 (65), 65 (24), 51 (12), 43 (25), 39 (20) and 32 (12).

*2-Hydroxy-2-ethyl-2-phenylacetonitrile $C_{10}H_{11}NO$ **2g***

^{1}H NMR ($CDCl_3$): δ 1.04 (t, 3H, CH_3); 1.98–2.18 (qq, 2H, CH_2); 2.90 (s, 1H, OH); 7.37–7.45 (m, 3H, H_{arom}); 7.55–7.58 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 8.79 and 36.89 (CH_3); 75.48 (C); 120.71 (CN); 2*125.07, 2*128.96, 129.29 and 139.78 (C_{arom}). MS: m/z 161 (M, 20), 132 (100), 105 (40), 77 (30), 51 (20) and 39 (6).

*2-Hydroxy-2-(dichloromethyl)-2-phenylacetonitrile $C_9H_7Cl_2NO$ **2h***

^{1}H NMR ($CDCl_3$): δ 3.94 (s, H, OH); 5.79 (s, 1H, CH); 7.45–7.50 (m, 3H, H_{arom}); 7.66–7.68 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$): δ 22.86 (CH); 76.01 (C); 126.41 (CN); 128.95, 130.67, 133.79 and 138.50 (C_{arom}). MS: m/z 219 (M+1, 0.1), 217 (M−1, 0.5), 215 (M−3, 1.0), 163 (0.5), 144 (1), 132 (45), 125 (5), 105 (100), 77 (50), 51 (26) and 39 (5).

*2-Hydroxy-2,5-dimethyl hexanenitrile $C_8H_{15}NO$ **2i***

^{1}H NMR ($CDCl_3$): δ 0.95 (d, 6H, CH_3); 1.10–1.90 (m, 7H); 1.61 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 22.42 (2*CH_3); 27.72, 27.95, 33.05, 39.72 (CH_2) 68.86 (C), 121.96 (CN). MS: m/z 140 (M−1, 0.3), 126 (3), 108 (10), 99 (12), 83 (5), 81 (14), 71 (100), 58 (34), 56 (54), 43 (88) and 41 (6).

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