Lomary Singh Moon (2009). ***New biocatalyst for nitrile hydrolysis and novel chiral solvating agents for NMR enantiodiscrimination of cyanohydrins.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.
Supervisor: Dr. R.S.Jolly **Acc. No. : Th-218**

SUMMARY OF THE THESIS

INTRODUCTION

Chirality is a key factor in the efficacy of many drugs and agrochemicals.[1] Natural predisposition for shape and handedness in molecular binding by receptors, pumps, and enzymes has been recognized as essential principle for effective drug design.[2] Increasing importance of chiral species in biological and pharmaceutical chemistry has resulted in a great surge of interest in (i) environment friendly and economically feasible methods for synthesis and (ii) development of accurate and convenient methods of measuring enantiomeric purity and absolute configuration of chiral compounds. This thesis describes the results of our efforts in targeting these two important issues. The thesis, for convenience, has been divided into two parts.

Part I, *new biocatalysts for nitrile hydrolysis*, deals with biocatalytic approach to nitrile hydrolysis.

Part II, *novel chiral solvating agents for NMR enantiodiscrimination of cyanohydrins*, deals with development of accurate and convenient methods of measuring enantiomeric purity and absolute configuration of cyanohydrins.

PART 1, CHAPTER 1: BIOCATALYZED NITRILE HYDROLYSIS: A REVIEW

Nitrile-converting enzymes comprise two distinct groups, nitrilases [EC 3.5.5.1] belonging to the 3rd class "hydrolases" and nitrile hydratases [EC 4.2.1.84] belonging to the 4th class "lyases". The enzymes, that hydrate nitriles into amides, are usually produced in bacteria along with an amidase activity, providing eventually carboxylates and ammonia. These enzymes are high-demand biocatalysts in drug synthesis, biodegradation and agricultural development.[3-6]

Although, a limited number of nitrilases are available to research laboratories, an impressive array of substrates has been studied for hydrolysis catalyzed by nitrilases and nitrile hydratases. Most of the reviews written on this topic have focused on classification of enzyme and/or of substrates. In the present review, we have made an attempt to cover maximum number of substrates that have been studied. We have presented a comprehensive list of substrates studied and products formed along with conversion, yield, e.e. and source of enzyme in a tabulated format. Thus, the table has multiple entries extracted from each published paper

depending upon the number of substrates reported in the paper. A section each in the review has been included for (i) hydrolysis of symmetrical and non-symmetrical dinitriles to cyanocarboxylic acids and (ii) hydrolysis of nitrile containing labile functional groups. In addition, the review covers recent literature on occurrence of nitrilases and nitrile hydratases in microorganisms. Mechanism of hydrolysis by a nitrilase and a nitrile hydratase has been briefly discussed.

PART 1, CHAPTER 2: BIOCATALYZED NITRILE HYDROLYSIS: RESULTS AND DISCUSSION

Biocatalytic nitrile hydrolysis is an attractive alternative to drastic chemical methods, not only in terms of environmental concerns but also in economics of the product formation. But in spite of the great synthetic potential that nitrilases offer, their utilization as a versatile biocatalyst is largely unexploited.[6] The scarcity of suitable and well-characterized nitrile-converting biocatalysts is a barrier to their application.[7] Nitrile-converting enzymes are generally not commercially available, are currently limited in variety (and therefore catalytic application), are usually labile, and are prone to product or substrate inhibition. These prevent their wider industrial application and therefore there is a constant demand for new nitrilases. Although few commercial processes that utilize nitrilase and nitrile hydratase exist, there are many opportunities for their industrial application, and hence many companies (such as DuPont, Lonza, Dow, Diversa, BASF, and DSM) have been investigating the application of nitrile biocatalysis for various processes. We started this project with objective to isolate and characterize a biocatalyst for nitrile hydrolysis with diverse industrial applications.

In this section, we have described (i) isolation of new biocatalysts for the nitrile hydrolysis, (ii) purification and characterization of the enzyme responsible for activity, (iii) substrate specificity profile of the biocatalyst and (iv) applications of the biocatalyst in preparation of intermediates of bioactive compounds and fine chemicals.

548 Strains of microorganisms were screened for nitrile hydrolysis activity using mandelonitrile **(1)** as substrate and isolated one strain TSCS4 51, which produced mandelic acid **(2)** with desired conversion rate and high e.e. The strain TSCS4 51 was identified as *Alcaligenes faecalis subsp. parafaecalis* based on the biochemical characterization and 16S rDNA analysis.

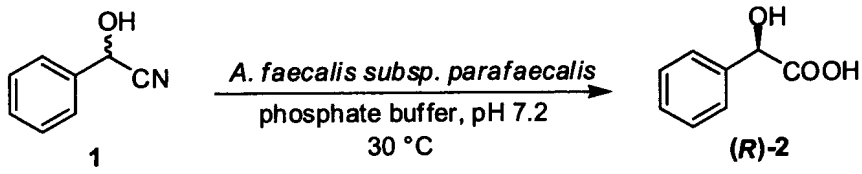
The enzyme responsible for activity has been purified by a 3-step procedure. Approximately 37-fold purification was required to obtain electrophoretically homogenous protein, which showed a single band at about ~46 kDa on SDS-PAGE run under reducing conditions. The pure protein hydrolyzed mandelonitrile **(1)** to mandelic acid **(2)**, no amide could be detected in the reaction mixture. Therefore, the enzyme was classified as nitrilase [EC 3.5.5.1]. The native molecular weight of nitrilase was calculated to be 546.38 kDa. Thus, nitrilase appears to be a homomeric aggregate of 12-subunits. The optimum pH and temperature for nitrilase was found to be 7.5 and 30 °C. N-terminal sequence, MQTRKIVRAAAVQAA was obtained by Edman degradation protocol.

***Alcaligenes faecalis subsp. parafaecalis* catalyzed conversion of racemic mandelonitriles to mandelic acids: dynamic kinetic resolution**

We started our investigation by studying in detail the *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis of mandelonitrile **(1)** to mandelic acid **(2)**. The reaction was done in phosphate buffer, pH 7.5 at 30 °C for 18 hr. The progress of the reaction was monitored by TLC. The product was purified by column-chromatography over silica-gel and characterized as mandelic acid **(2)** based on ^{1}H NMR, which showed presence of aromatic proton at δ 7.35-7.47 as multiplet and methine proton as singlet at δ 5.26. The ^{13}C NMR spectrum was also in agreement with assigned structure. E.e. was determined by using optically pure (1*R*,2*R*)-1,2–diphenylethane-1,2-diamine as chiral shift reagent.[8] The (*R*)-configuration was assigned based on comparison of chemical shift values in ^{1}H NMR of standard (*R*)- and (*S*)-mandelic acid obtained in presence of chiral shift reagent. The (*R*)-configuration was further confirmed by comparing optical rotation with literature value, $[\alpha]_D^{25}$ = -154.2° (c = 1, H_2O); lit.[9] $[\alpha]_D^{25}$ = -155° (c = 1, H_2O).

Theoretically, kinetic resolution can lead to a maximum of 50% conversion to produce product of high enantiomeric purity. But, *A. faecalis subsp. parafaecalis* catalyzed reaction proceeded to 100% conversion. The product was isolated in 92.3% yield and >99% e.e.; thus leading to much desired dynamic kinetic resolution. The results of *A. faecalis subsp. parafaecalis* catalyzed conversion of mandelonitrile to (*R*)-(-)-mandelic acid **(2)** are summarized in Scheme 1.

Scheme 1



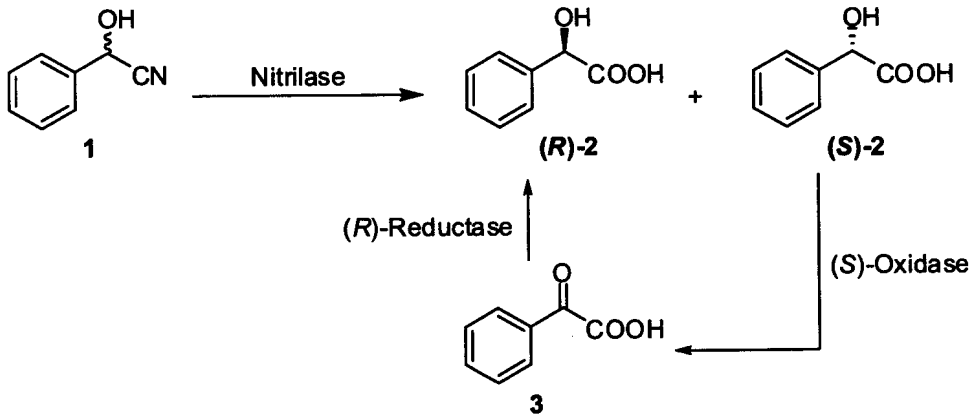
1 (*R*)-2

Conversion rate = 13.1 µmoles/min./g wet cell mass
Conversion = 100%
Yield = 92.3%
E.e. = >99%

Probable mechanism for dynamic kinetic resolution of mandelonitrile to mandelic acid catalyzed by *Alcaligenes faecalis subsp. parafaecalis*

Alcaligenes faecalis subsp. parafaecalis was able to oxidize 2-phenylethanol to acetophenone. Based on this observation, we explored a possible mechanism for dynamic kinetic resolution as shown in Scheme 2.

Scheme 2



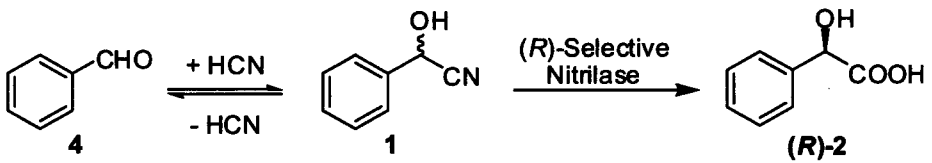
1 (*R*)-2 (*S*)-2 3

When racemic mandelic acid **(2)** was incubated with *A. faecalis subsp. parafaecalis*, e.e of mandelic acid increased from 0 to 78.26% in 1 hr and >99% in 5 hr. However, there was concomitant formation of benzoic acid and 2-oxophenylacetic acid **(3).** When (*S*)-mandelic acid **(2)** was subjected to similar conditions, no significant change in e.e. was observed, but formation of benzoic acid and 2-oxophenylacetic acid **(3)** occurred as revealed by ^{1}H NMR. However, (*R*)-mandelic acid **(2)** treated analogously did not show any change in e.e. or degradation to benzoic acid or 2-oxophenylacetic acid **(3)**. These results clearly show that deracemization of racemic

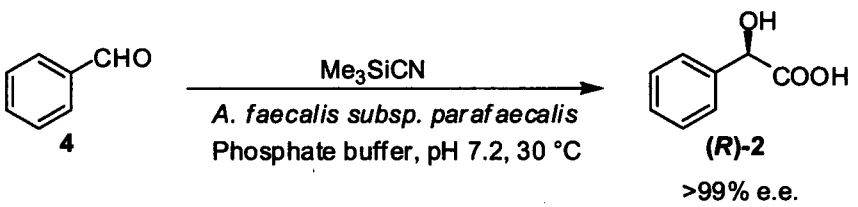
mandelic acid has occurred due to selective degradation of (*S*)-mandelic acid **(2)** and not by selective oxidation-reduction. However, this mechanism is unlikely to operate for dynamic kinetic resolution of mandelonitrile as the yield of isolated product, (*R*)-**2** was 92.3%.

An alternate mechanism for dynamic kinetic resolution is shown in Scheme 3. According to this mechanism, equilibrium exists between mandelonitrile and benzaldehyde. Whereas, (*R*)-mandelonitrile **(1)** is rapidly hydrolyzed to (*R*)-mandelic acid **(2)** by (*R*)-selective nitrilase, (*S*)-mandelonitrile **(1)** undergoes racemization. The production of (*R*)-mandelic acid **(2)** as a sole product from *A. faecalis subsp. parafaecalis* catalyzed reaction of benzaldehyde **(4)** with trimethylsilyl cyanide (Scheme 4) provides evidence in the favour of this mechanism. This mechanism is also consistent with literature report.[9]

Scheme 3



Scheme 4



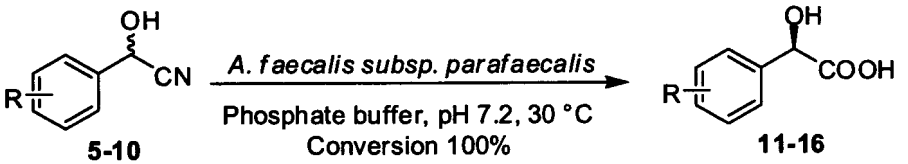
***Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis of 2-, 3- and 4-methoxymandelonitrile (5-7) and chloromandelonitrile (8-10) (Scheme 5)**

Mandelic acid and its derivatives are a commercially important class of α-hydroxy acids.[10] A range of mandelonitriles **(5-10)** were subjected to *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis. In all these examples reaction proceeded to 100% conversion. Enantiomeric excess was determined by ^{1}H NMR method using a new chiral solvating agent developed by us and discussed in Part II, Chapter 2 of this thesis. Whereas, 3-methoxymandelonitrile **(6)** produced corresponding mandelic acid in excellent e.e. of >99%, 2-

methoxymandelonitrile **(5)** and 4-methoxymandelonitrile **(7)** produced corresponding mandelic acids with moderate e.e. of 94.6% and 85.6%, respectively. Absolute configuration was assigned as *R* in all cases based on comparison of sign of optical rotation with literature value. 2-methoxymandelic acid **(11)** $[\alpha]_D^{25}$ = -117.3° (c = 0.3, EtOH); lit.[11] $[\alpha]_D^{25}$ = -124° (c = 0.3, EtOH), 3-methoxymandelic acid **(12)** $[\alpha]_D^{25}$ = -116.4° (c = 0.3, EtOH); lit. [12] $[\alpha]_D^{25}$ = -117° (c = 0.3, EtOH)) and 4-methoxymandelic acid **(13)** $[\alpha]_D^{25}$ = -120.7° (c = 0.3, H_2O); lit.[12] $[\alpha]_D^{25}$ = -141° (c = 0.3, H_2O).

Alcaligenes faecalis subsp. parafaecalis catalyzed hydrolysis of 2-chloromandelonitrile **(8)** resulted in formation of (*R*)-2-chloromandelic acid **(14)** in 89.7% yield and >99% e.e. The absolute configuration was assigned based on comparison of optical rotation with literature value; $[\alpha]_D^{25}$ = -157.2° (c = 0.3, EtOH); lit.[12] $[\alpha]_D^{25}$ = -158° (c = 0.3, EtOH). 3-Chloromandelonitrile **(9)** produced (*R*)-3-chloromandelic acid **(15)** in 91.1% and yield 97.2% e.e. The absolute configuration was assigned based on comparison of optical rotation with literature value; $[\alpha]_D^{25}$ = -112.6° (c = 4, EtOH); lit.[12] $[\alpha]_D^{25}$ = -116° (c = 4, EtOH). Similary, 4-chloromandelonitrile **(10)** gave (*R*)-3-chloromandelic acid **(16)** in 87.2% yield and 98.3% e.e. The absolute configuration was assigned based on comparison of optical rotation with literature value; $[\alpha]_D^{25}$ = -131.7° (c = 0.3, EtOH); lit.[12] $[\alpha]_D^{25}$ = -134° (c = 0.3, EtOH).

Scheme 5



5-10 **11-16**

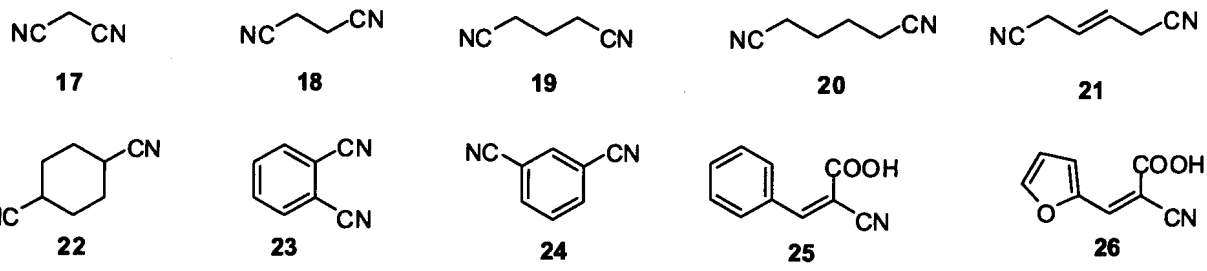
Entry	Substrate	Product	Yield %	E.e. %
1	2-OCH_3 **(5)**	2-OCH_3 **(11)**	89.5	94.6
2	3-OCH_3 **(6)**	3-OCH_3 **(12)**	90.6	>99
3	4-OCH_3 **(7)**	4-OCH_3 **(13)**	88.8	85.6
4	2-Cl **(8)**	2-Cl **(14)**	89.7	>99
5	3-Cl **(9)**	3-Cl **(15)**	91.1	97.2
6	4-Cl **(10)**	4-Cl **(16)**	87.2	98.3

Alcaligenes faecalis subsp. parafaecalis catalyzed conversion of dinitriles to cyanocarboxylic acid

Cyanocarboxylic acids are important intermediates for a variety of applications. An attractive approach to the synthesis of this type of important compounds is the selective hydrolysis of dinitriles to cyanocarboxylic acids. However, there are currently no non enzymatic catalysts available, which could hydrolyze only one nitrile group of a dinitrile with high selectivity at complete conversion of the dinitrile.

Desymmetrization of symmetrical dinitriles

Alcaligenes faecalis subsp. parafaecalis catalyzed hydrolysis of a range of diverse symmetrical dinitriles was studied. In all examples studied, exclusive hydrolysis of one nitrile group occurred. Prolonging the reaction time or subjecting the isolated cyanocarboxylic acids to fresh cells of *Alcaligenes faecalis subsp. parafaecalis* did not result in the hydrolysis of second nitrile. Therefore, the reaction is highly selective and barring few exceptions conversion rate of 100% was achieved with most substrates. The cyanocarboxylic acids obtained by desymmetrization reaction catalyzed by *Alcaligenes faecalis subsp. parafaecalis* are shown in Figure 1. Malononitrile **(17)**, succinonitrile **(18)**, glutaronitrile **(19)**, adiponitrile **(20)**, (*E*)-hex-3-enedinitrile **(21)**, cyclohexane-1,4-dicarbonitrile **(22)**, Benzene-1,2-dicarbonitrile **(23)**, benzene-1,3-dicarbonitrile **(24)** failed to undergo any reaction; the starting material was recovered unchanged from the reaction mixture. However, 2-benzylidenemalononitrile **(25)** and 2-(furan-2-ylmethylene)malononitrile **(26)** on similar treatment produced benzoic acid and furoic acid in 63.7 and 73.9% yield, respectively instead of the desired cyanocarboxylic acids.



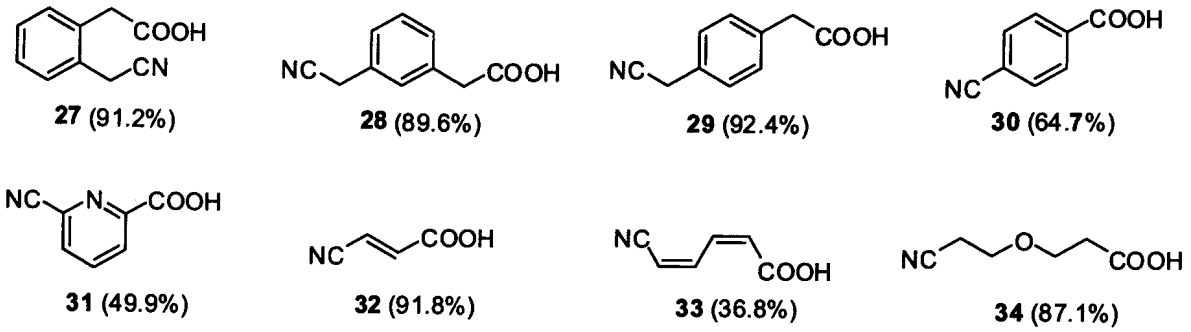


Figure 1 Cyanocarboxylic acids obtained from corresponding dinitriles by desymmetrization reaction catalyzed by *Alcaligenes faecalis subsp. Parafaecalis*. The yield of isolated product is given in parenthesis.

Alcaligenes faecalis subsp. parafaecalis catalyzed regioselective hydrolysis of dinitriles

4-(Cyanomethyl)benzonitrile **(35)** on *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis produced 2-(4-cyanophenyl)acetic acid **(36)** in 92.3% yield (Entry 1, Table 1). Similarly, 2-(cyanomethyl)benzonitrile **(37)** produced 2-(2-cyanophenyl)acetic acid **(38)** in 90.7% yield (Entry 2, Table 1). 4-fluorobenzene-1,2-dicarbonitrile **(39)** and pyridine-3,4-dicarbonitrile **(40)** failed to undergo any hydrolysis (Entry 3 and 4, Table 1). The starting material was recovered unchanged from these reactions. 2-methylenepentanedinitrile **(41)** produced 4-cyanopent-4-enoic acid **(42)** in 91.4% yield as the sole product (Entry 5, Table 1). Similarly, 4-cyanopentanoic acid **(43)** produced 2-methylpentanedinitrile **(44)** in 66.1% yield (Entry 6, Table 1).

Hydrolysis of nitriles by pure nitrilase of *Alcaligenes faecalis subsp. parafaecalis*

All the substrates gave similar results as were obtained when whole cells of *Alcaligenes faecalis subsp. parafaecalis* were used as biocatalyst, except substrates **18-21**. Whereas, whole cells of *A. faecalis subsp. parafaecalis* failed to hydrolyse these substrates, reaction with pure enzyme resulted in formation of corresponding cyanoacid **45-48** (Entry 1 to 4, Table 2) The structure of all products was confirmed by NMR spectroscopy.

Table 1 *Alcaligenes faecalis subsp. parafaecalis* catalyzed regioselective hydrolysis of dinitriles

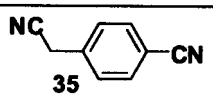
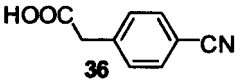
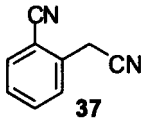
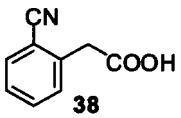
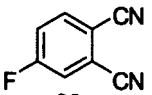
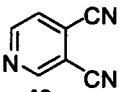
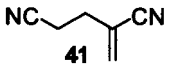
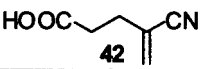
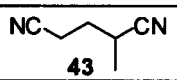
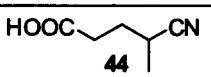
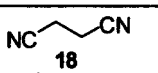
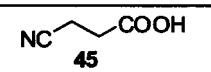
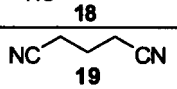
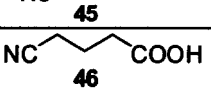
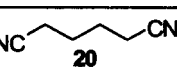
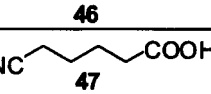
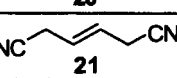
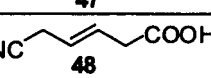
Entry	Substrate and Spectral data	Product and Spectral data	Conversion (Yield)
1	 **35**	 **36**	100% (92.3%)
2	 **37**	 **38**	100% (90.7%)
3	 **39**	No Reaction	-
4	 **40**	No Reaction	-
5	 **41**	 **42**	100% (91.4%)
6	 **43**	 **44**	79.8% (66.1%)

Table 2 Hydrolysis of aliphatic dinitriles with pure nitrilase of *Alcaligenes faecalis subsp. parafaecalis*

Entry	Substrate and Spectral data	Product and Spectral data	Conversion (Yield)
1	 **18**	 **45**	73.7% (61.1%)
2	 **19**	 **46**	49.0% (38.4%)
3	 **20**	 **47**	64.9% (51.3%)
4	 **21**	 **48**	100% (86.2%)

Application of nitrilase of *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis of nitriles in preparation of intermediates of bioactive compounds and fine chemicals

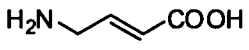
Clopidogrel is an oral antiplatelet agent to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. It is marketed under the trade name Plavix with sales of US$5.9 billion in 2005. (*R*)-2-chloromandelic acid **(14)** is a key intermediate

for synthesis of Clopidogrel. *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis of 2-chloromandelonitrile **(8)** resulted in formation of (*R*)-2-chloromandelic acid **(14)** in 89.5% yield and >99% e.e (Scheme 5).

Amibegron (SR-58,611A) and Solabegron (GW-427,353) are selective agonist for the β3 adrenergic receptor. Amibegron is the first orally active β_3 agonist developed that is capable of entering the Central Nervous System, and has antidepressant and anxiolytic effect,[13, 14] while Solabegron is being developed for the treatment of overactive bladder and irritable bowel syndrome.[15, 16] (*R*)-3-chloromandelic acid **(15)**, a key intermediate for the synthesis of Amibegron and Solabegron was obtained by *A. faecalis subsp. parafaecalis* catalyzed hydrolysis of 3-chloromandelonitrile **(9)** in 91.1% yield and 97.2% e.e. (Scheme 5).

Picamilon and GABA can be easily synthesized from 3-cyanopropanoic acid **(45)**. Selective hydrolysis of succinonitrile **(18)** with nitrilase of *Alcaligenes faecalis subsp. parafaecalis* resulted in formation of 3-cyanopropanoic acid **(45).** *Trans*-4-aminocrotonic acid **(49)** and 5-Aminopentanoic acid **(50)** are efficient GABA analog/homologue.[17] Selective nitrile hydrolysis of fumaronitrile **(51)** and glutaronitrile **(19)** by *Alcaligenes faecalis subsp. parafaecalis*, resulted in the formation of (E)-3-cyanoacrylic acid **(32)** and 4-cyanobutanoic acid **(46)**, respectively, which can be easily converted to *trans*-4-aminocrotonic acid **(49)** and 5-aminopentanoic acid **(50)**, respectively.

Azafenidin is a herbicide developed by DuPont. 5-cyanovaleramide **(52)** is an intermediate in the synthesis of Azafenidin.[18] As shown above, selective nitrile hydrolysis of adiponitrile **(20)** with nitrilase of *Alcaligenes faecalis subsp. parafaecalis* results in the formation of 5-cyanovaleric acid **(47)** (Table 2)



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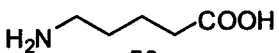

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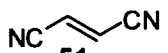

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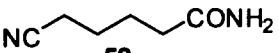

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2-methylglutaronitrile **(43)** (a byproduct produced during the manufacture of adiponitrile **(20)** for nylon-6,6) was efficiently converted into 4-cyanopentanoic acid **(44)** by regioselective nitrile hydrolysis by *Alcaligenes faecalis subsp. parafaecalis* (Scheme 5). **44** is a precursor for

the preparation of 1,5-Dimethyl-2-piperidone (Xolvone), a precision cleaning solvent currently in commercial development by DuPont for use in a variety of industrial applications.

In conclusion, a nitrilase, which converted racemic mandelonitrile **(1)** to (*R*)-(-)-mandelic acid **(2)** in >99% e.e. and 100% conversion has been purified from *Alcaligenes faecalis subsp. parafaecalis*. The enzyme is made up of 12 homomeric subunits of ~46KDa and showed maximum activity at pH 7.5 and temperature 30 ^{0}C. The enzyme exhibited excellent enantio and regioselectivity with a range of substrate tested. Moreover, desymmetrization of dinitrile to cyanocarboxylic acid, which is extremely difficult by chemical methods, was achieved in near 100% efficiency. Preparation of key intermediates of several commercially important molecules by *Alcaligenes faecalis subsp. parafaecalis* catalyzed hydrolysis of nitriles was successfully achieved.

PART 2: CHAPTER 1: AN OVERVIEW

Excellent reviews are already available on NMR methods for the determination of e.e. and absolute configuration.[19-24] In the present review, we have restricted ourselves to providing basic, but essential information for methods involving chiral derivatizing agents (CDAs) and chiral solvating agents (CSAs). An attempt has been made to tabulate available reagents and their application with respect to various functional groups. Known literature on NMR methods for determination of e.e and absolute configuration specific to cyanohydrins has been reviewed in the last section of the Chapter.

PART 2; CHAPTER 1: NOVEL CHIRAL SOLVATING AGENTS: RESULTS AND DISCUSSION

Section 1

The ability of a host molecule to form geometrically different diastereomeric complexes with antipods of a chiral compound has been exploited for the discrimination of enantiomers by NMR.[19-24] While rigid structures with either built-in cages[25-31] or with strong conformational bias[31-35] have been amongst the most used hosts for NMR enantiodiscrimination, structurally flexible simpler structures[36-38] have also been shown to discriminate enantiomers in NMR with similar resolutions through a wrap-around action mode. In this section we have shown that a

much simpler structure, an ion-pair of (*R*) or (*S*)-mandelate and dimethylamminopyridinium ions possesses structural features, which are sufficient for NMR enantiodiscrimination of cyanohydrins. Moreover, ^{1}H NMR data of cyanohydrins of known configuration obtained in presence of mandelate-dimethylaminopyridinium ion-pair point to the existence of a correlation between chemical shifts and absolute configuration of cyanohydrins.

Assigning the absolute configuration of cyanohydrins is not just an extension of the procedure for secondary or tertiary alcohols because of complicating effects of cyano group. Even though cyanohydrins keep, at a first glance, some resemblance with the structure of secondary or tertiary alcohols, the presence of the strongly polar -CN substituent makes the geminal hydroxynitrile moiety a wholly novel situation from the structural point of view to which the NMR procedures previously described for secondary alcohols cannot be applied without a previous and rigorous validation.[39]

Based on literature reports (i) that a mixture of DMAP and carboxylic acid exists as ion-pair in chloroform,[40] (ii) that pyridinium cations interact with anions through moderate to strong NH$\cdots$O bond[41] and (iii) second oxygen of carboxylate in ion-pair is available as H-bond acceptor,[42] we purposed a hypothetical model for ternary complex of mandelonitrile with mandelate-DMAPH$^+$ ion-pair (Figure 2). The α-H in complex A and R-group in complex B of cyanohydrin faces phenyl group of mandelate; thereby opening a possibility for NMR enantiodiscrimination of mandelonitrile by mandelate-DMAPH$^+$ ion-pair.

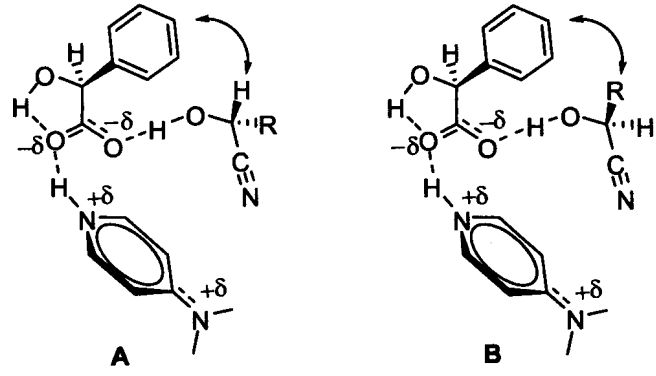


Figure 2 Hypothetical model showing ternary complexes of (*R*)-cyanohydrin and (*S*)-cyanohydrin with (*S*)-mandelate-DMAPH$^+$ ion-pair. H in complex A and R-group in complex B of cyanohydrin faces phenyl group of mandelate

To test this hypothesis, we recorded ^{1}H NMR of racemic mandelonitrile (30 mM) in the presence of 1 mol equivalent each of (*S*)-mandelic acid and DMAP in $CDCl_3$. We were pleased to note that α-H of two enantiomers of racemic mandelonitrile appeared as well resolved singlets ($\Delta\Delta\delta$=0.084 ppm) in approximate 1:1 ratio based on integral values. These results clearly show that mandelate-DMAPH$^+$ ion-pair is an effective chiral solvating agent for mandelonitrile. Since base-line separation of α-H of two enantiomers of racemate has occurred, e.e. of mandelonitrile can be obtained from integral values of α-H.

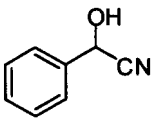
NMR enantiodiscrimination of a variety of racemic cyanohydrins derived from aliphatic and aromatic aldehydes and ketones with (*S*)-mandelate-dimethylaminopyridinium ion-pair was studied (Figure 3). Excellent base line separation occurred in all cases with $\Delta\Delta\delta$ in the range of 0.055 to 0.112 ppm for all aldehyde cyanohydrins studied. The baseline separation occurred, even when the methine proton appeared as a doublet, triplet or quartet. Excellent baseline separation coupled with the fact that resolved singlets for aldehyde cyanohydrins appeared in the least complex region of ^{1}H NMR suggested that mandelic acid/DMAP mixture can be used for the accurate determination of the optical purity of these compounds.

The $\Delta\Delta\delta$ for ketone cyanohydrins was of the order of 0.010 to 0.031 ppm. The magnitude of resolution for ketone cyanohydrins was less compared to aldehyde cyanohydrins, but sufficient for the determination of optical purity of these compounds.

Enantiodiscrimination of cyanohydrins: Determination of absolute configuration

Having demonstrated enantiodiscrimination of cyanohydrins by mandelate-DMAPH$^+$ ion-pair, the next goal was to investigate the suitability of the method for the determination of their absolute configuration. NMR spectrum behaviour of a series of cyanohydrins of known absolute configuration was studied using (*R*)- or (*S*)-mandelic acid, in combination with DMAP, to find the existence of any correlation between the absolute configuration and the NMR chemical shifts. $\Delta\delta^{RS}$ values, obtained with (*R*)- and (*S*)-mandelic acid, respectively, for several aldo- and ketocyanohydrins of known configuration are shown in Figure 4. Aldehyde cyanohydrins **(*R*)-1, 7, 53, 10, 56, 60** and **61**,which have same spatial relationship showed a

positive $\Delta\delta^{RS}$ value, whereas cyanohydrins **(*S*)-54, 55, 57, 58** and **59** with opposite spatial arrangement, showed negative $\Delta\delta^{RS}$ values. Similarly, ketone cyanohydrins, **(*R*)-62, 64, 65** and **67,** which are configurationally related to **(*R*)-1** showed positive $\Delta\delta^{RS}$ values, whereas cyanohydrins, **63, 66** and **68,** which are configurationally related to **(*S*)-54** showed negative $\Delta\delta^{RS}$ values. Moreover, the enantiomers of **(*R*)-1, 7, 53, 60, 62** and **64,** which are configurationally, related to **(*S*)-54,** exhibited negative $\Delta\delta^{RS}$ values. Similarly, the enantiomers of **(*S*)-55** and **57,** which are configurationally, related to **(*R*)-1** showed positive $\Delta\delta^{RS}$ value. Thus, $\Delta\delta^{RS}$ sign is characteristic for this enantiomeric series and can be used for the assignment of absolute configuration. However, $\Delta\delta^{RS}$ value for pyridine compound **(*R*)-56** was poor compared to other aldehyde cyanohydrin series. Since basic nitrogen on pyridine can alter the nature of complex formation, assignment of absolute configuration in pyridine series may not be reliable.



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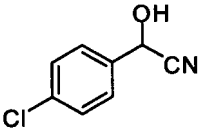

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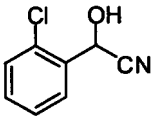

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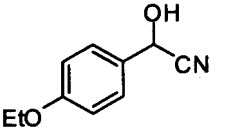

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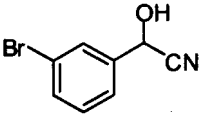

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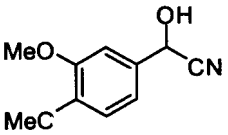

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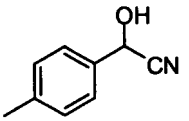

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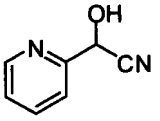

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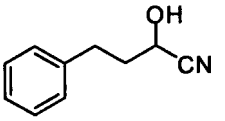

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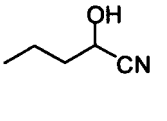

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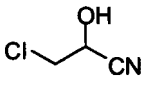

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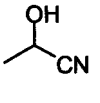

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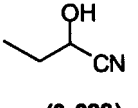

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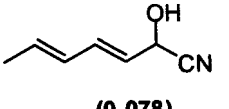

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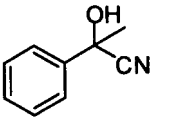

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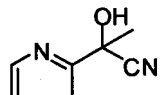

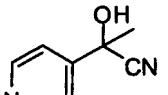

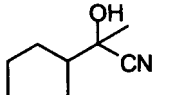

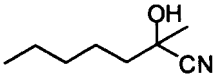

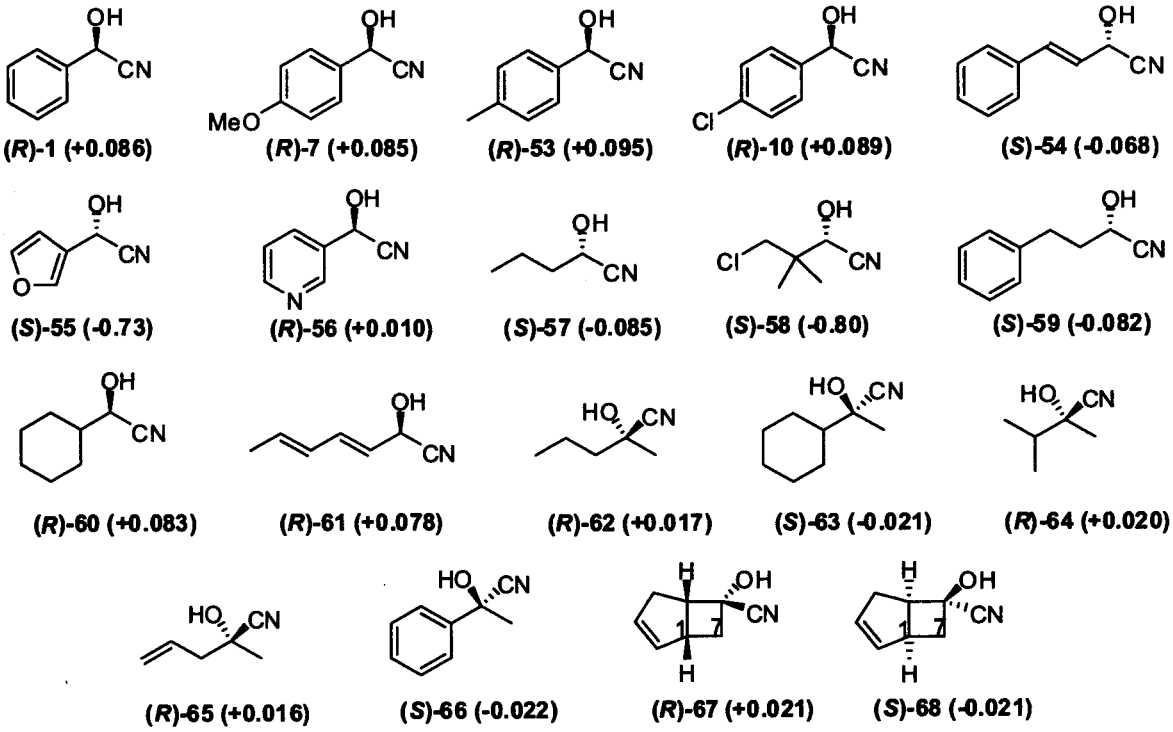

Figure 4 Correlation between chemicals shifts and absolute configuration of cyanohydrins. $\Delta\delta^{RS}$ ($\Delta\delta^{R}$-$\Delta\delta^{S}$) values are shown in parenthesis.