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(54) Title: IMPROVED SYNTHESIS OF OPTICALLY PURE (S) - 3-CYANO-5-METHYL-HEXANOIC ACID ALKYL ESTER, AN INTERMEDIATE OF (S)- PREGABALIN

(57) Abstract: The present invention is directed towards synthesis of (S) - 3-cyano-5-methyl-hexanoic acid ethyl ester. A cost effective, eco-friendly process for preparation of enantiomerically pure (S)-3-cyano-5-methyl-hexanoic acid alkyl ester, intermediate of γ-amino acids, particularly (S)-pregabalin.

WO 2011/141923 A2

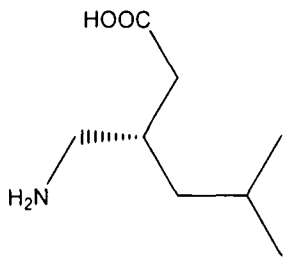
# IMPROVED SYNTHESIS OF OPTICALLY PURE (S) - 3-CYANO-5-METHYL-HEXANOIC ACID ALKYL ESTER, AN INTERMEDIATE OF (S)- PREGABALIN

## Field of the Invention:

The invention relates to a cost effective, eco-friendly process for preparation of enantiomerically pure (*S*)-3-cyano-5-methyl-hexanoic acid alkyl ester, intermediate of γ-amino acids, particularly (*S*)-pregabalin.

## Background of the Invention:

(*S*)-3-(Aminomethyl)-5-methylhexanoic acid [CAS No. 148553-50-8], which is also known as β-isobutyl-γ- aminobutyric acid, isobutyl-GABA, or pregabalin [I] is a potent anticonvulsant. As discussed in U.S. Patent No. 5,563,175, pregabalin exhibits anti-seizure activity and is found to be useful for treatment of various other conditions, like pain, fibromyalgia, physiological conditions associated with psychomotor stimulants, inflammation, gastrointestinal damage, insomnia, alcoholism and various psychiatric disorders, including mania and bipolar disorder. (U.S. Patent No. 6,242,488; U.S. Patent No. 6,326,374; U.S. Patent No. 6,001,876; U.S. Patent No. 6,194,459; U.S. Patent No. 6, 329, 429; U.S. Patent No. 6, 127,418; U.S. Patent No. 6,426, 368; U.S. Patent No. 6,306,910; U.S. Patent No. 6,359,005).



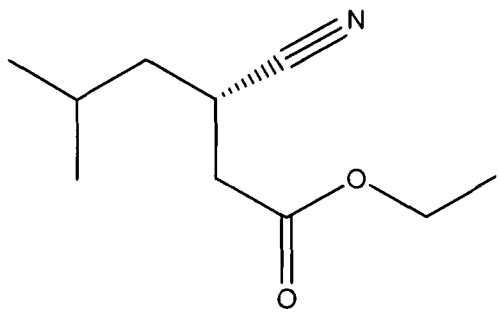

[I]

A number of synthetic schemes have been developed for preparation of pregabalin. Typically, a racemic mixture of 3-aminomethyl-5-methylhexanoic acid has been synthesized and subsequently resolved into (*R*) and (*S*) enantiomers. Processes for

synthesis of racemic [I] have been developed through an azide intermediate, a malonate intermediate or a nitrile intermediate.

(*S*)-3-Cyano-5-methyl-hexanoic acid ethyl ester [II] is one of the key intermediates for the synthesis of (*S*)-pregabalin. A number of approaches for synthesis of racemic as well as enatiomerically pure compound [II] are reported in the literature. However, majority of processes have used potassium cyanide for introduction of cyano function during synthesis of compound [II].

The relevant literature on synthesis of compound [II] is briefed hereinafter.




[II]

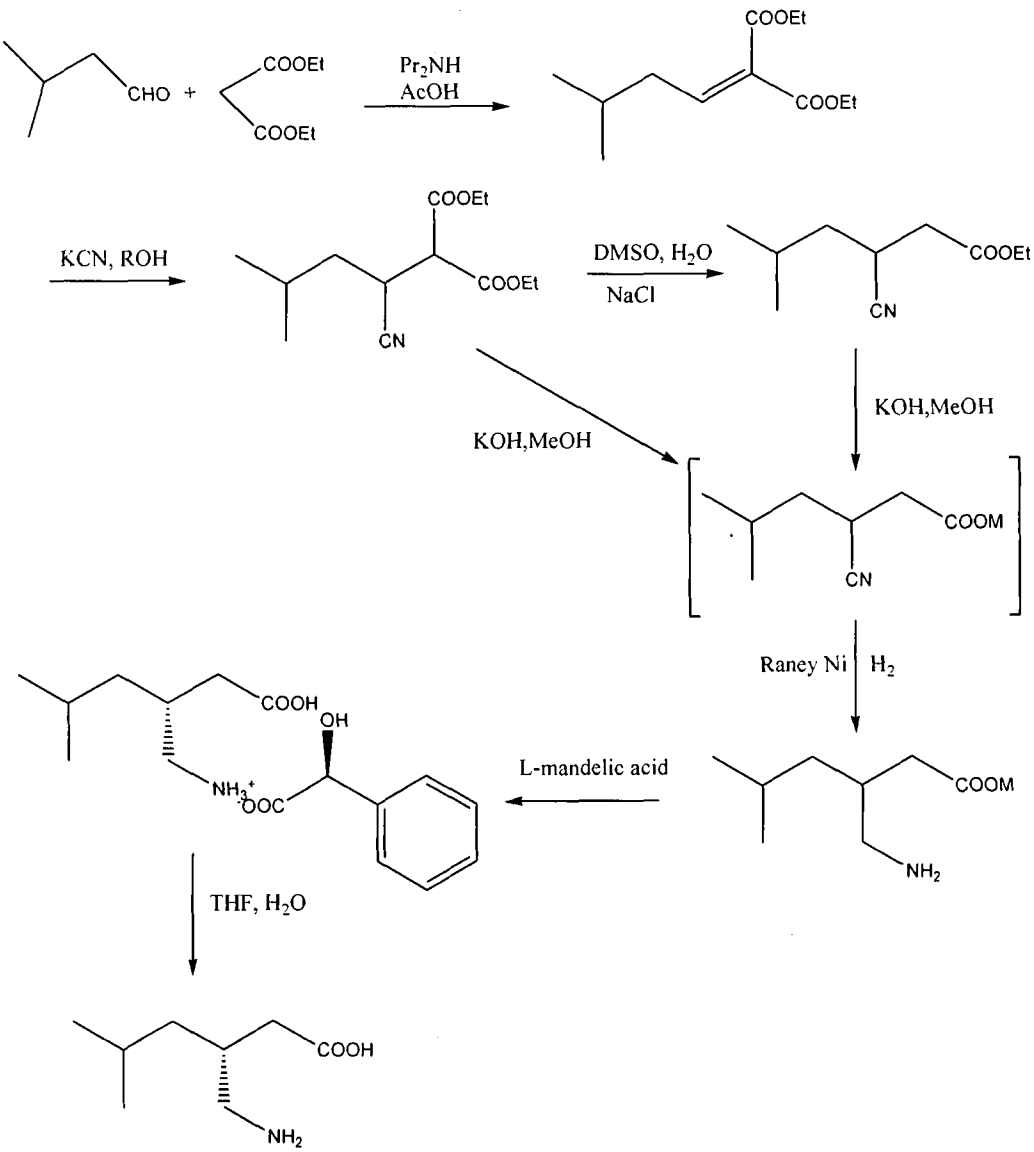
Scheme 1 depicts the process for preparation of (*S*)-pregabalin as disclosed in US patent No. 5,637,767. In this process *iso*-valeraldehyde is condensed with diethyl malonate in presence of base to obtain 2-carboxyethyl-5-methylhex-2-enoic acid ethyl ester.

α,β unsaturated, 2-carboxyethyl-5-methylhex-2-enoic acid ethyl ester on Michael addition of potassium cyanide yields racemic 3-cyano-2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester.

Further decarboxylation of racemic 3-cyano 2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester in DMSO/NaCl gives the 3-cyano-5-methylhexanoic acid ethyl ester

which is subsequently hydrogenated to obtain racemic pregabalin, and further resolved with (*S*)-mandelic acid to obtain (*S*)-pregabalin [I].

Although the above method provides *(S)*-pregabalin in high optical purity, the overall yield is very poor. Furthermore, the process uses potassium cyanide which is very toxic and hazardous, and requires special precaution for handling, specifically in plant scale manufacturing.




**Scheme 1**

Scheme 2 gives the reaction scheme for synthesis of (*S*)-pregabalin as described in the process disclosed in US patent publication No. 2005/0283023 A1.

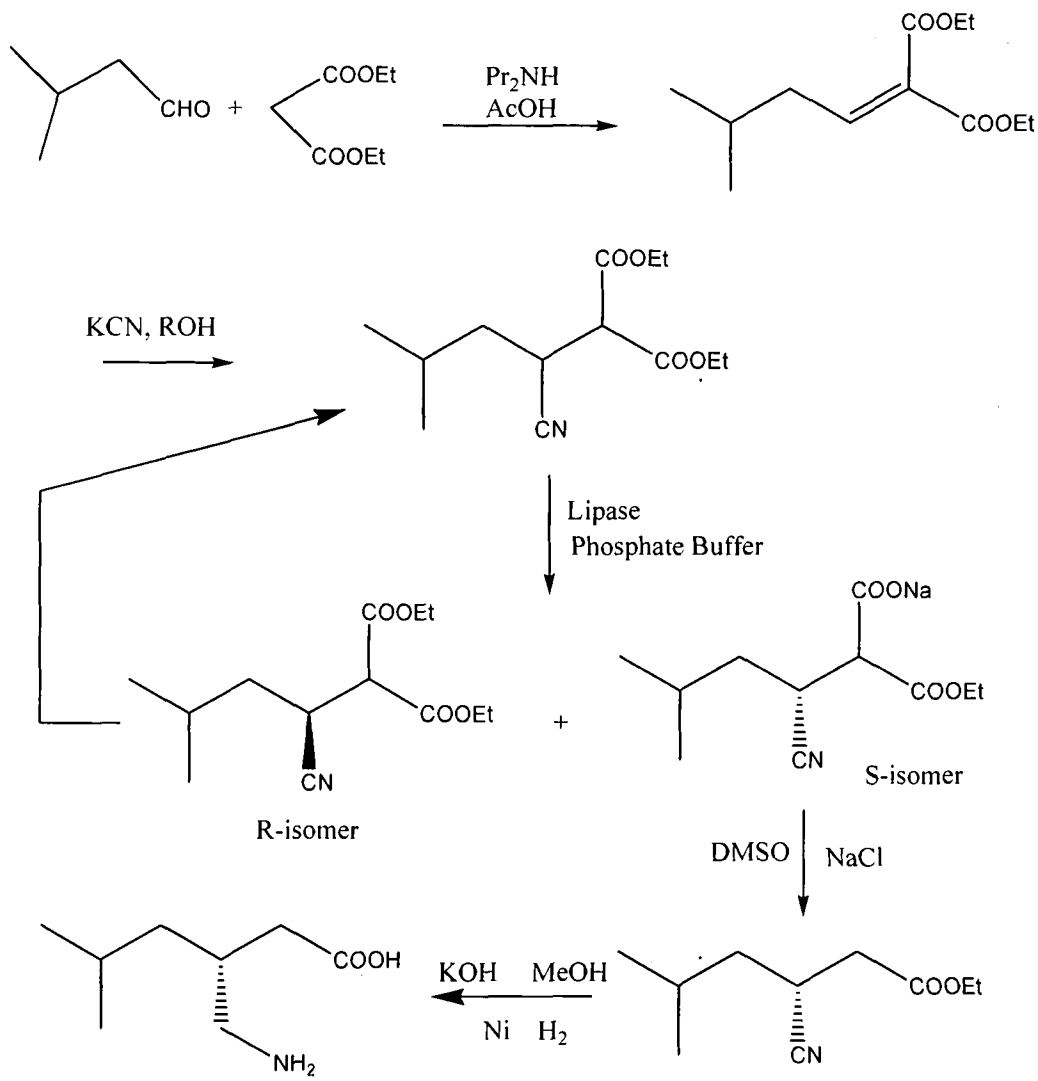
In this process, 2-carboxyethyl-5-methylhex-2-enoic acid ethyl ester intermediate is obtained through Knoevenagel condensation of *iso*-valeraldehyde with diethyl malonate in presence of base.

Michael addition of potassium cyanide on α,β unsaturated 2-carboxyethyl-5-methylhex-2-enoic acid ethyl ester yields the 3-cyano 2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester.

Subsequently resolution of 3-cyano 2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester through enzyme is carried out to obtain optically pure (*S*)-3-cyano-2-ethoxycarbonyl 5-methyl hexanoic acid sodium salt and (*R*)-3-cyano-2-ethoxycarbonyl-5-methylhexanoic acid ethyl ester.

(*S*)-3-Cyano-2-ethoxycarbonyl-5-methyl hexanoic acid sodium salt upon decarboxylation gives the (*S*)-3-cyano-5-methylhexanoic acid ethyl ester and subsequently is hydrogenated to obtain (*S*)- pregabalin.

The use of potassium cyanide in the above process is one of the major drawbacks for this scheme. Moreover, yield of the enzymatic reaction is poor which leads to the increase in over all cost of the process.




**Scheme 2**

Scheme 3 depicts the process for synthesis of (*S*)-Pregabalin as documented in US patent application No. 2003/0212290 A1.

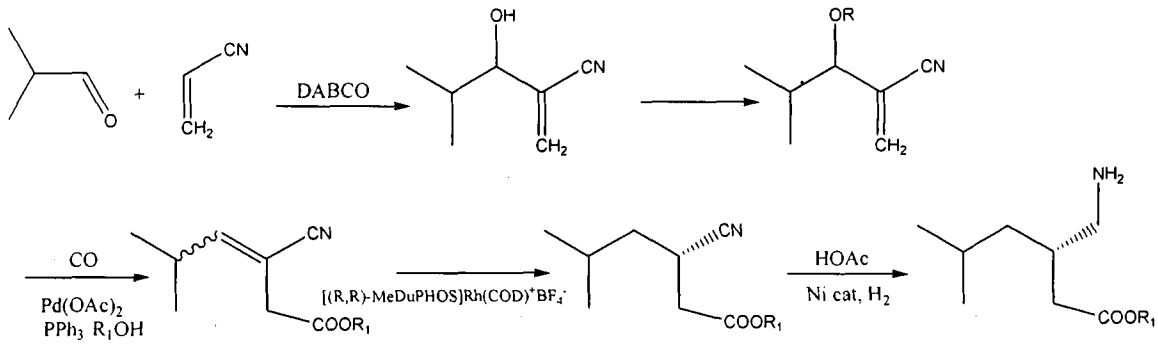
In the said process, 3-hydroxy-4-methyl-2-methylenepentanitrile is prepared through Baylis-Hillman reaction of *iso*-butyraldehyde with acrylonitrile.

The Baylis-Hillman adduct is further carbonylated with carbon monoxide to yield unsaturated cyano intermediate, which on subsequent asymmetric hydrogenation gives the *(S)* - 3-cyano-5-methylhexanoic acid ethyl ester.

However, the disclosed method requires the use of carbon monoxide under high pressure, raising considerable problem during scale-up of the process to commercial scale.

The said application discloses the use of various $C_2$ symmetric bisphosphine ligands, including *(R, R)* Me-DUPHOS, which is very costly and isolation of catalyst is very difficult. The "turn over" number of catalyst is not satisfactory, which results in significant impact on the final cost of the product.

Furthermore, the disclosed method requires the use of carcinogenic acrylonitrile and the use of highly toxic carbon monoxide under high pressure.

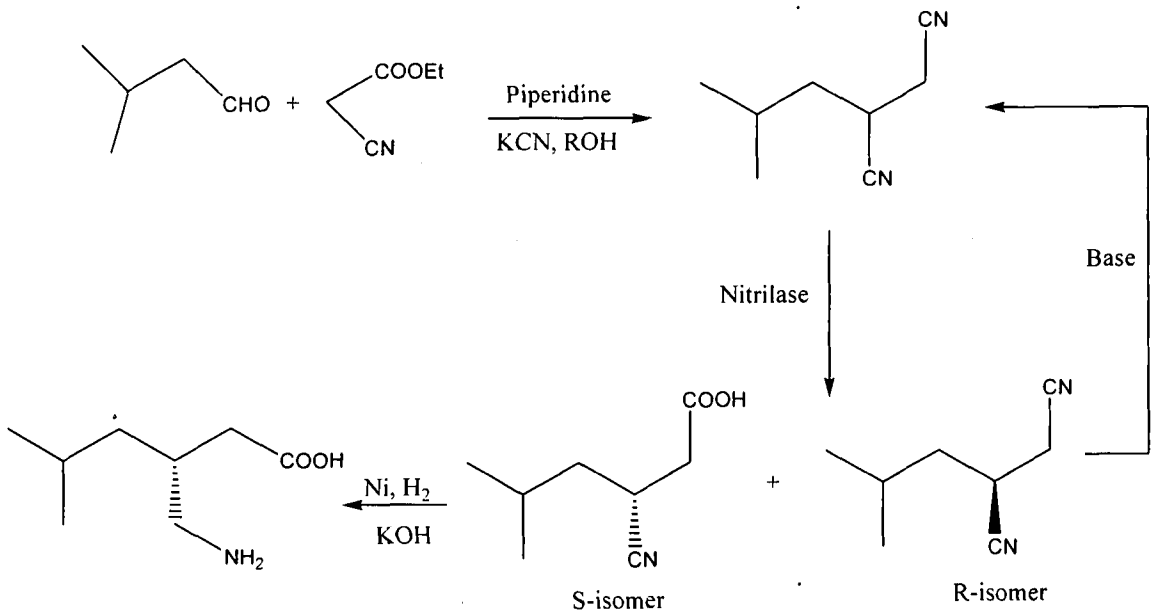



**Scheme 3**

Scheme 4 represents the process for preparation of *(S)*-pregabalin as disclosed in US patent application No. 2007/0196905 A1.

The said method uses the enzymatic, *i.e.* Nitrilase catalyzed stereo-selective reduction of (*RS*)-2-isobutyl succinonitrile to produce (*S*)-3-cyano-5-methylhexanoic acid ethyl ester, which is further reduced to obtain *(S)*-pregabalin.

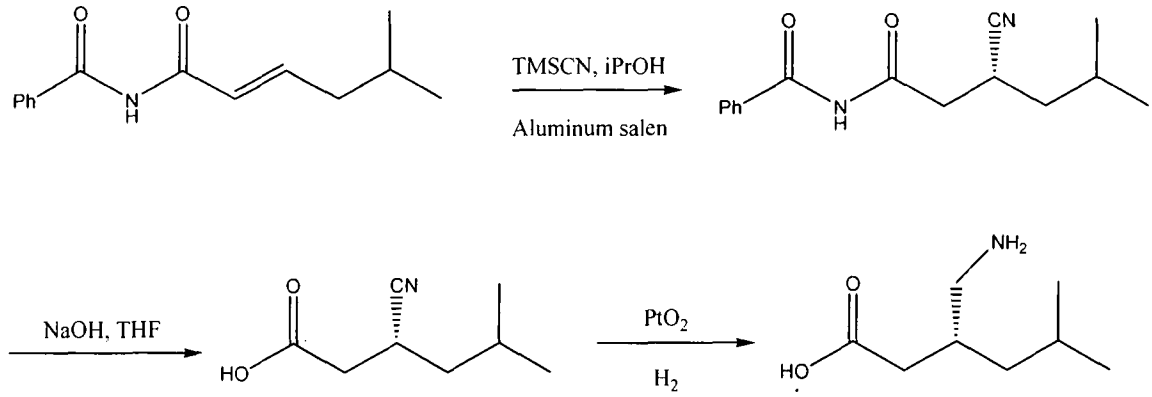
Enantiomeric purity of final product is good; however, yield with enzymatic reaction is poor and process uses potassium cyanide to yield one of the intermediate thus making the process not desirable at industrial scale.




**Scheme 4**

Scheme 5 depicts the process disclosed by G. M. Sammis *et al.* (J. Am. Chem. Soc., 2003, 125(15) 4442-43) where an aluminum salen catalyst is used in the conjugate addition of hydrogen cyanide to α,β-unsaturated imides.

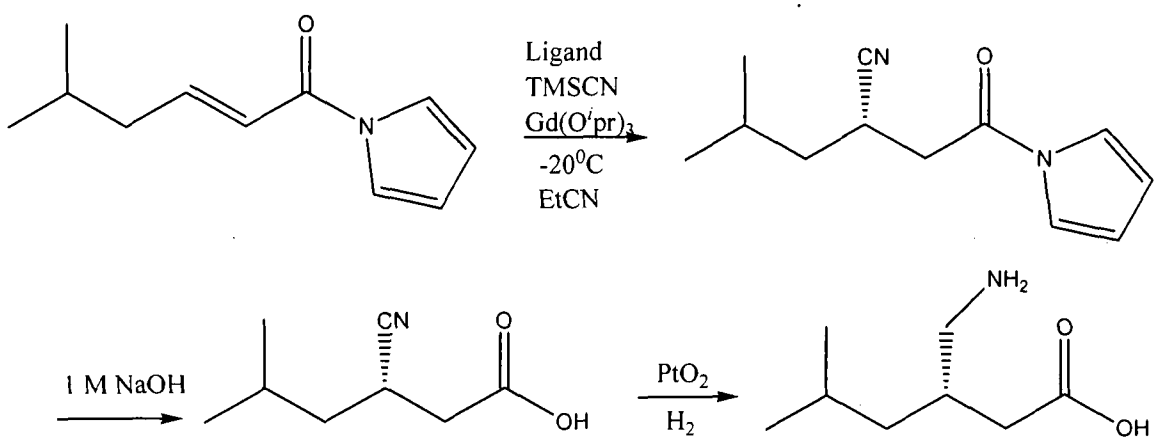
This process is also not practical for large scale production due to the use of highly poisonous chemicals namely trimethylsilyl cyanide, and use of aluminum salen catalyst, which is costly thereby creating significant impact on the final cost of the product.




**Scheme 5**

Scheme 6 represents the process described in EP No. 1,995,250. Here, complex asymmetric ligand is used for the conjugate addition of hydrogen cyanide equivalent to α,β-unsaturated imides which is carried out in presence of a Gadolinium catalyst.

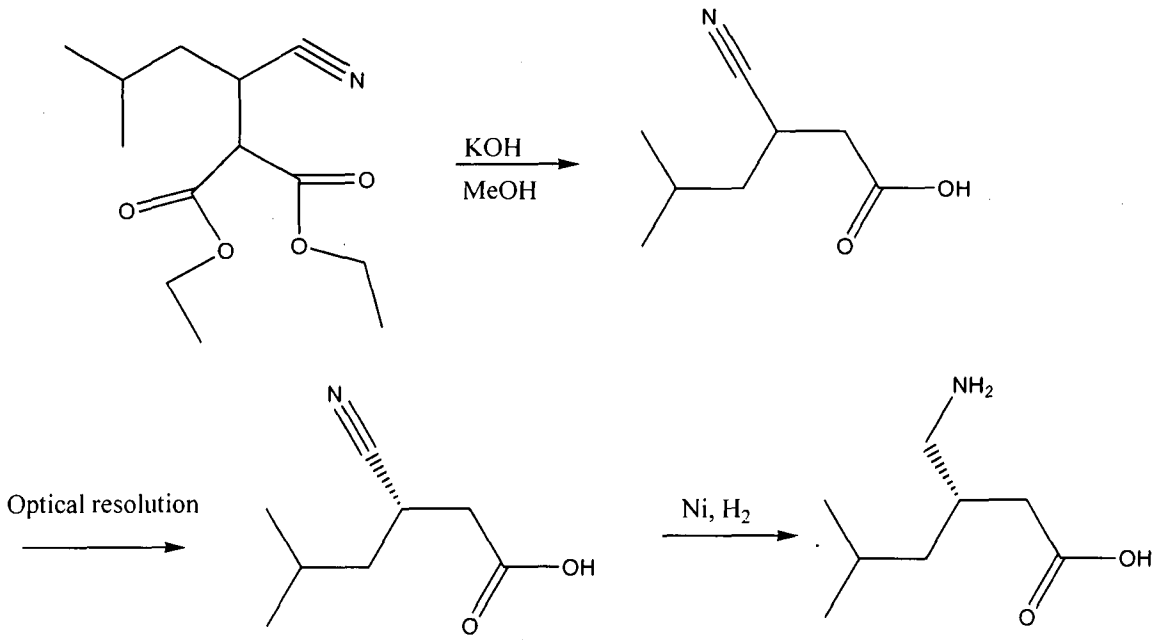
The preparation of ligand is tedious and requires a number of synthetic steps, hence the high cost. This process is also not practical for large scale production due to the use of highly poisonous sodium cyanide equivalent.




**Scheme 6**

WO2007/143152 A2, reports the optical resolution of (*S*)-3-cyano-5-methylhexanoic acid through diastereomeric salt formation with optically pure amines. Optically pure (*S*)-3 – cyano -5-methylhexanoic acid is further reduced to obtain *(S)*-pregabalin.

However, synthesis of 3-cyano-2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester intermediate requires use of potassium cyanide. Reaction scheme is depicted in Scheme 7.




**Scheme 7**

It is evident from prior art that the backbone in the manufacture of pregabalin is synthesis of a crucial intermediate "(*S*) - 3-cyano-5-methyl-hexanoic acid or its ester" and the processes reported in the literature for its synthesis are not very attractive in view of cost efficiency, use toxic reagents and eco-hazardous operations. Hence, there is a need for an eco-friendly, "green", cost effective, easy-to-operate, industrial-

scale synthesis of (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester and/or its intermediate 2-((*S*)-1-cyano-3-methyl-butyl)-malonic acid diethyl ester.

This invention provides an improved, highly cost effective, operation friendly, green process for the title compound.

**Objects of the Invention:**

Hence the object of this invention is to provide lipase catalyzed resolution of (*RS*) - 3-cyano-5-methyl-hexanoic acid ethyl ester or enantiomerically enriched (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester to optically pure (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester, having excellent yield and high optical purity (98-99%).

Another object of the present invention is synthesis of the novel compound diethyl 2-cyano-2-isobutylsuccinate through a novel method and further conversion of it to (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester.

Yet another object of the present invention is to provide novel cost effective and green synthetic methodology for the synthesis of (*RS*)-3-cyano-5-methyl-hexanoic acid ethyl ester via Stobbe condensation of dibenzyl succinate with *iso*-butyraldehyde.

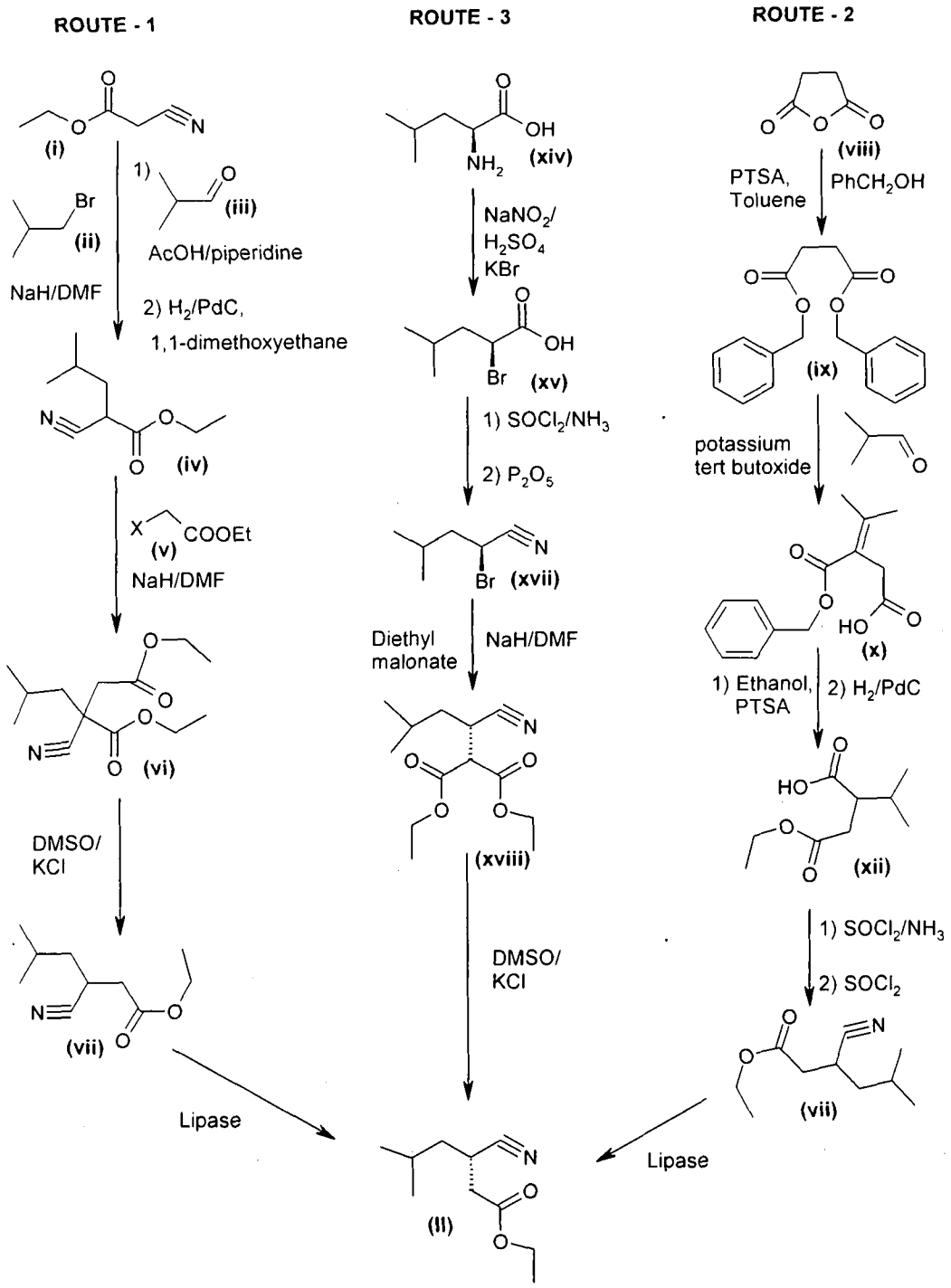
Further object of the present invention is to provide a novel method for synthesis of enantiomerically enriched (*S*)-3-cyano-2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester from *S*-Leucine, an intermediate for the title compound (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester.

It is also an important object of the present invention to provide a process for recycling of undesired enantiomer *i.e.* (*R*)-3-cyano-5-methyl-hexanoic acid via converting into corresponding ester, followed by racemization to (*RS*) - 3-cyano-5-

methyl-hexanoic acid ethyl ester, which could be reused for enzymatic resolution, thereby improving the atom economy and hence cost.

**Summary of the Invention:**

The present invention is directed towards synthesis of (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester. The invention is summarized below in scheme A.




SCHEME - A

The invention comprise of

**A) The processes for preparation of (*RS*)-3-cyano-5-methyl-hexanoic acid alkyl ester, preferably ethyl ester.**

This has been achieved through two novel routes:

**Route 1**: Cyanoacetic acid ethyl ester (i) was condensed with 1-bromo-2-metnyl propane (ii) in presence of sodium hydride in an organic solvent such as N,N-dimethyl formamide to give 2-cyano-4-methyl-pentanoic acid ethyl ester (iv). Compound (iv) was also prepared by condensation of 2-methyl-propionaldehyde (iii) with cyanoacetic acid ethyl ester (i) in presence of piperidinium acetate, and further hydrogenation using palladium charcoal catalyst. Compound (iv) was further reacted with halo acetic acid ethyl ester (v) in presence of sodium hydride in an organic solvent such as N,N-dimethylformamide to give diethyl 2-cyano-2-isobutylsuccinate (vi). Compound (vi) was then treated with potassium chloride in organic solvent such as dimethylsulfoxide to get 3-cyano-5-methyl-hexanoic acid ethyl ester (vii), which was resolved to (*S*) 3-cyano-5-methyl-hexanoic acid ethyl ester (II) using Lipase.

**Route 2**: Dihydrofuran-2,5-dione (viii) was treated with benzylalcohol in presence of *para*-toluenesIfonic acid (PTSA) to give succinic acid dibenzyl ester (ix). Compound ix was further treated with 2-methyl-propionaldehyde and potassium tertiary butoxide to give 2-isopropylidene-succinic acid 1-benzyl ester (x), which on treatment with *para*-toluenesulfonic acid and ethanol gave 2-isopropylidene-succinic acid 1-benzyl ester 4-ethyl ester (xi). Compound of formula (xi) on hydrogenation using palladium on charcoal catalyst gave 2-isopropyl-succinic acid 4-ethyl ester (xii). Compound (xii) was further treated with thionyl chloride to give acid chloride which was treated in situ with ammonia to get 3-carbamoyl-5-methyl-hexanoic acid ethyl ester (xiii). Compound (xiii) on dehydration in presence of thionylchloride gave 3-cyano-5-methyl-hexanoic acid ethyl ester (vii), which was resolved to (*S*) 3-cyano-5-methyl-hexanoic acid ethyl ester (II) using Lipase.

**B) The process for the preparation of enantiomerically enriched 3-cyano-5-methyl-hexanoic acid alkyl ester, preferably ethyl ester.**

**Route 3**: (*S*)-2-Amino-4-methyl-pentanoic acid [*S*-leucine] (xiv) was treated with sodium nitrite and sulfuric acid followed by treatment of reaction mass with potassium bromide gave (*S*)-2-bromo-4-methyl-pentanoic acid (xv). Compound (xv) was further treated with thionyl chloride to get acid chloride which on treatment with ammonia gave (*S*)-2-bromo-4-methyl-pentanoic acid amide (xvi). Dehydration of compound of formula (xvi) in presence of phosphorous pentoxide gave (*S*)-2-bromo-4-methyl-pentanenitrile (xvii). Compound (xvii) on $S_N2$ displacement reactions with diethyl malonate yielded enantiomerically pure 2-((*S*)-1-cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii), which upon decarboxylation in presence of potassium chloride and dimethylsulfoxide gave enantiomerically pure (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester (II).

**C) Kinetic resolution of (*RS*)-3-cyano-5-methyl-hexanoic acid alkyl ester or enantiomerically enriched (*S*) 3-cyano-5-methyl-hexanoic acid alkyl ester to obtain optically pure (*S*)- 3-cyano-5-methyl-hexanoic acid alkyl ester**

Resolution of racemic 3-cyano-5-methyl-hexanoic acid ethyl ester using lipase as provided in scheme B and C effected as follows - Stereo-selective enzymatic hydrolysis of (*RS*)-3-cyano-5-methyl-hexanoic acid alkyl ester (vii) or enantiomerically enriched 3-cyano-5-methyl-hexanoic acid alkyl ester was carried out to obtain optically pure (*S*) - 3-cyano-5-methyl-hexanoic acid alkyl ester (II) and the hydrolyzed acid of the undesired isomer *i.e.* (*R*)-3-cyano-5-methyl-hexanoic acid (III) was converted back to racemic compound (vii) and reused for further enzymatic resolution to improve over all atom economy and process efficiency.

**Brief Description of Accompanying Drawings**

Figure 1: Rate of formation of *(R)* - 3-cyano-5-methylhexanoic acid and *(S)* - 3-cyano-5-methylhexanoic acid Vs. time at 25 $^0$C

Figure 2: Rate of formation of *(R)* - 3-cyano-5-methylhexanoic acid and *(S)* - 3-cyano-5-methylhexanoic acid Vs. time at 15 $^0$C

**Detailed Description of the Invention:**

This invention provides

i) Lipase catalyzed resolution of (*RS*) - 3-cyano-5-methyl-hexanoic acid ethyl ester or enantiomerically enriched (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester to optically pure (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester, having excellent yield and high optical purity (98-99%).

ii) Synthesis of the novel compound diethyl 2-cyano-2-isobutylsuccinate (vi) through a novel method as an intermediate for the title compound.

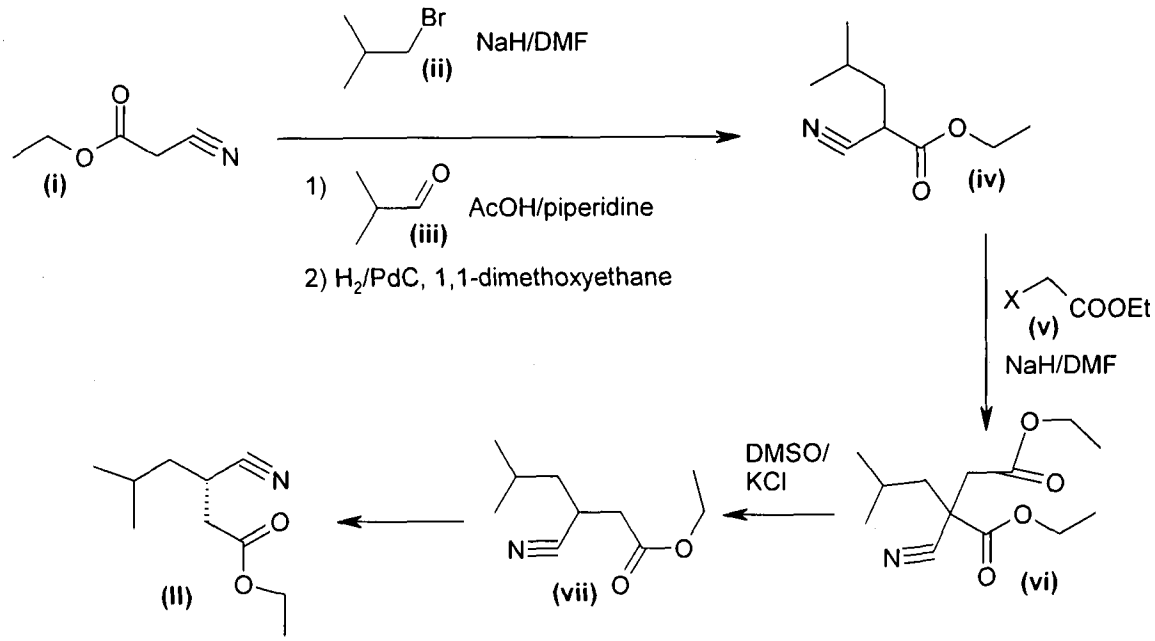
iii) Novel cost effective and green synthetic methodology for the synthesis of (*RS*)-3-cyano-5-methyl-hexanoic acid ethyl ester via Stobbe condensation of dibenzyl succinate with *iso*-butyraldehyde.

iv) A novel method for synthesis of enantiomerically enriched (*S*)-3-cyano-2-ethoxycarbonyl-5-methyl-hexanoic acid ethyl ester from *S*-Leucine , an intermediate for the title compound (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester.

v) Recycling of undesired enantiomer *i.e.* (*R*)-3-cyano-5-methyl-hexanoic acid via converting into corresponding ester, followed by racemization to (*RS*) - 3-cyano-5-methyl-hexanoic acid ethyl ester, which could be reused for enzymatic resolution, thereby improving the atom economy and hence cost.

**A) The processes for preparation of (*RS*)-3-cyano-5-methyl-hexanoic acid alkyl ester, preferably ethyl ester. This has been achieved through two novel routes**

**Route 1:** Scheme B depicts the reaction scheme for the preparation of (*RS*)-3-cyano-5-methyl-hexanoic acid ethyl ester from ethyl cyano acetate.




**Scheme - B:** Route 1

According to one aspect, the present invention provides the process for the preparation of racemic or enantiomerically enriched *S* isomer of 3-cyano-5-methyl-hexanoic acid ethyl ester [compound (vii) or (II)] from compound of formula (i).

In one aspect, compound (iii) on reaction with compound (i) in presence of base and subsequent hydrogenation to gives compound (iv).

Further, compound (iv) on reaction with $XCH_2COOR$ (X=halide excluding F) in polar and non-polar solvents in presence of base gives compound (vi), which is further decarboxylated to obtain compound (vii).

Knoevenagel condensation of *iso*-butyraldehyde (iii) with ethyl cyanoacetate (i) in presence of base yields the compound (iv) (J. Am. Chem. Soc. 1944, 66, 886-888).

Typically, compound (ii) on reaction with compound (i) in presence of base to yield an unsaturated intermediate which on subsequently hydrogenated in polar solvent, in presence of a noble metal catalyst under hydrogen pressure to obtain compound (iv).

Noble metal catalyst can be selected from platinum oxide, palladium on carbon, Raney nickel and palladium hydroxide on carbon; preferably the noble metal catalyst is palladium on carbon and palladium hydroxide on carbon.

Polar solvent may be selected from 1,4-dioxane, tetrahydrofuran, dimethoxy ethane, and diglyme; preferably dimethoxy ethane and diglyme.

Generally, hydrogen pressure is about 1 $kg/cm^2$ to 5 $kg/cm^2$; preferably 2 $kg/cm^2$.

After the completion of reaction, reaction mixture is filtered through filtrate pad to remove the catalyst. Solvent is distilled out to obtain compound (iv).

Compound (iv) is also obtained by condensation reaction with *iso*-butyl bromide (ii) with compound (i) in presence of base in polar solvent.

Polar solvent may be selected from 1,4-dioxane, tetrahydrofuran, dimethoxy ethane, and diglyme; preferably dimethoxy ethane and diglyme.

Compound (vi) is obtained by reacting compound (iv) with halo-acetic acid ester in presence of base in polar solvents.

Halide in halo acetic acid ester may be chloro, bromo and iodo; preferably chloro and bromo; more preferably bromo.

Base used for synthesis of compound (vi) was selected from alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate; preferably sodium hydride.

Polar solvent may be selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4-dioxane, and dimethoxy ethane; preferably dimethoxy ethane and N, N-dimethyl formamide; more preferably dimethoxy ethane and dimethyl sulphoxide.

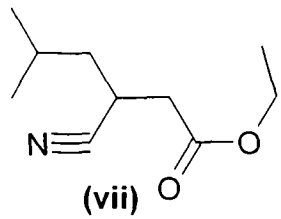
Compound (vi) is usually obtained by carrying out the reaction at temperature of about 10 to 80 $^0$C; preferably at 50 to 60 $^0$C.

Compound (vii) is obtained from compound (vi) by decarboxylation in dimethyl sulfoxide with potassium chloride or sodium chloride; preferably potassium chloride.

Compound (vii) is usually obtained by conducting reaction at temperature of about 140 $^0$ to 180 $^0$C; preferably at 150 $^0$ to 160 $^0$C.

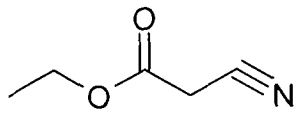
The above may be described as under:

A process for synthesis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (vii)



(vii)

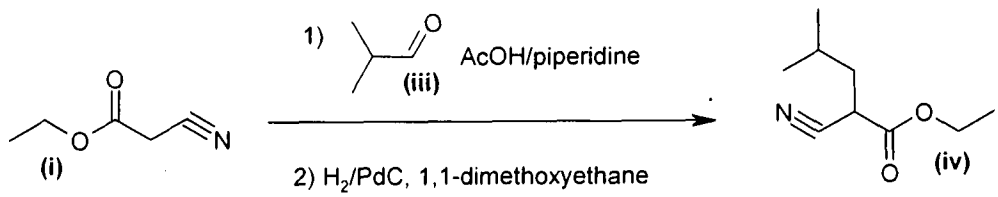
from ethyl cyano acetate of formula (i)



(i)

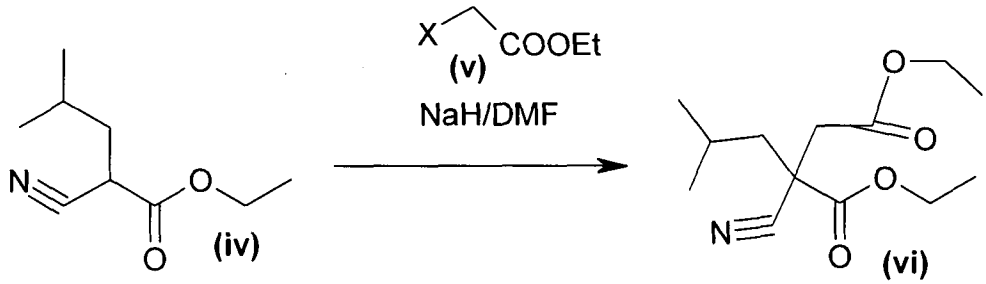
comprising,

a) condensation of 2-methyl-propionaldehyde with cyanoacetic acid ethyl ester in presence of base such as piperidinium acetate, and further hydrogenation using noble metal catalyst such as platinum oxide, palladium on carbon, Raney nickel and palladium hydroxide on carbon, preferably palladium on carbon and palladium hydroxide on carbon in polar solvent such as 1,4-dioxane, tetrahydrofuran, dimethoxy ethane and diglyme, preferably dimethoxy ethane and diglyme under hydrogen pressure of about 1 kg/cm$^2$ to 5 kg/cm$^2$, preferably about 2 kg/cm$^2$, further isolation of the product in solution form from the catalyst by filtration;

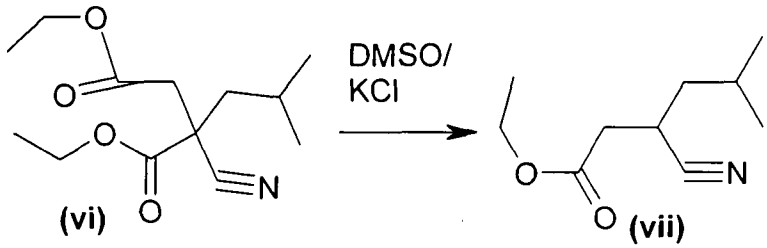



b) reaction of Compound of formula (iv) with halo acetic acid ethyl ester (v), wherein halo group include chloro, bromo and iodo, in presence of base such as alkali hydroxide, sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride, in a polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4-dioxane, and dimethoxy ethane, preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably

dimethoxy ethane and dimethyl sulphoxide at temperature of about 10 to 80 $^0$C, preferably at 50 to 60 $^0$C to give diethyl 2-cyano-2-isobutylsuccinate (vi);

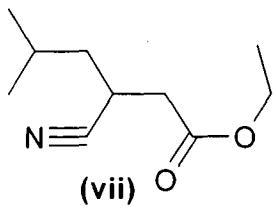



c) reaction of compound (vi) with potassium chloride or sodium chloride in an organic solvent such as dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get 3-Cyano-5-methyl-hexanoic acid ethyl ester (vii);

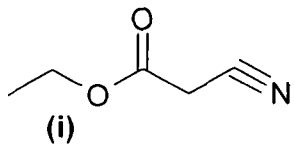



Such that at each step the intermediates were optionally isolated and purified with suitable process.

A process for synthesis of 3-cyano-5-methylhexanoic acid of formula (vii)



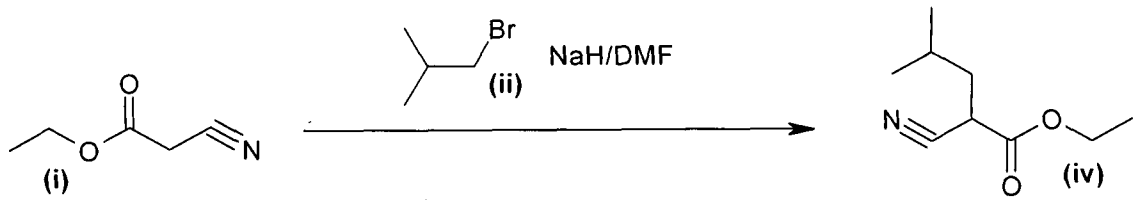

from ethyl cyano acetate of formula (i)



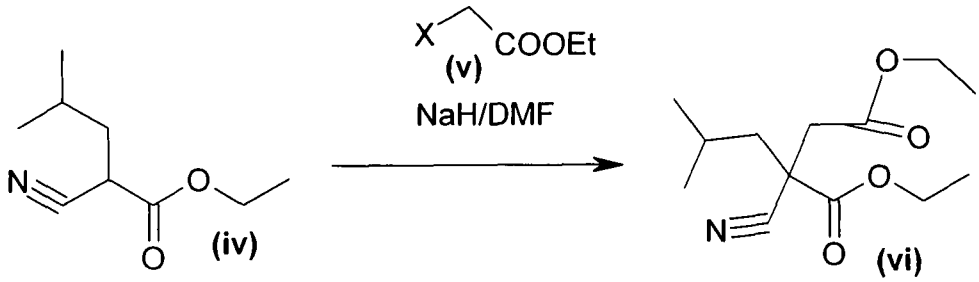
(i)

comprising,

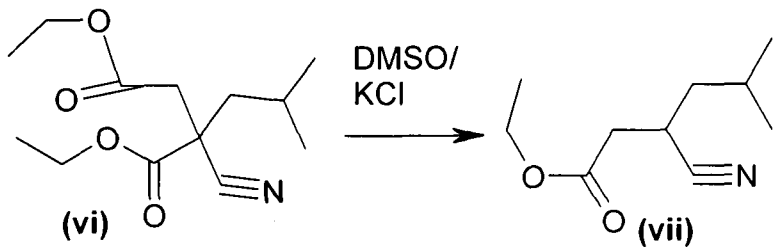
a) condensation of cyanoacetic acid ethyl ester with 1-bromo-2-methyl propane in presence of base such as alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride in a polar solvent such as 1,4-dioxane, tetrahydrofuran, dimethoxy ethane, and diglyme, preferably dimethoxy ethane and diglyme to give 2-cyano-4-methyl-pentanoic acid ethyl ester of formula (iv);




b) reaction of Compound of formula (iv) with halo acetic acid ethyl ester (v), wherein halo group include chloro, bromo and iodo, in presence of base such as alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride, in a polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4-dioxane, and dimethoxy ethane, preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably dimethoxy ethane and dimethyl sulphoxide at temperature of about 10 to 80 $^0$C, preferably at 50 to 60 $^0$C to give diethyl 2-cyano-2-isobutylsuccinate (vi);



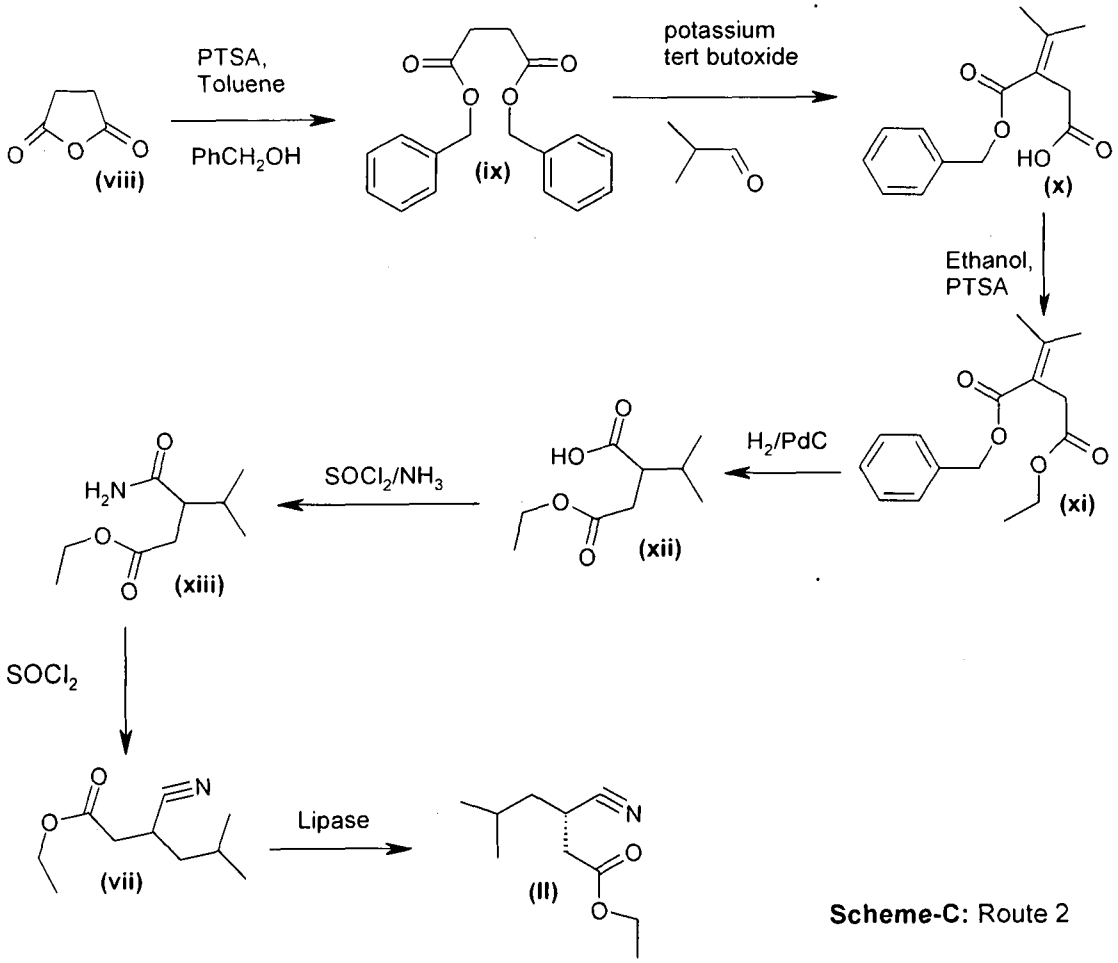

c) reaction of compound (vi) with potassium chloride or sodium chloride in an organic solvent such as dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get 3-Cyano-5-methyl-hexanoic acid ethyl ester (vii);




Such that at each step the intermediates were optionally isolated and purified with suitable process.

**Route 2:** Scheme C depicts the reaction scheme for the preparation of (*RS*)-3-cyano-5-methyl-hexanoic acid ethyl ester (vii) and further its resolution to *S*-isomer (II) from succinic anhydride.

According to another aspect, the present invention provides the process for the preparation of compound (vii) from compound (viii).




**Scheme-C:** Route 2

Compound (viii) is reacted with benzyl alcohol to obtain compound (ix) in presence of *p*-toluene sulphonic acid, which has not been hitherto reported.

Stobbe condensation of compound (ix) with *iso*-butyraldehyde in polar solvent in presence of a base yields the compound (x).

Polar solvent may be selected from *tert*-butanol, tetrahydrofuran, dimethyl sulfoxide and dimethoxy ethane; preferably *tert*-butanol.

Base is selected from potassium *tert*-butoxide, sodium hydride, sodium ethoxide, sodium methoxide; preferably potassium *tert*-butoxide.

Compound (x) is usually obtained by carrying out the reaction at temperature of about 25 to 80 $^0$C; preferably at 50 to 60 $^0$C.

The Stobbe adduct thus on acid catalyzed esterification with aliphatic alcohol produces the compound (xi).

Aliphatic alcohols may be selected from straight chain or branched, of $C_1$ to $C_6$ carbon chain length; preferably ethanol.

Hydrogenation and hydrogenolysis of compound (xi) in presence of noble metal catalysts produces compound (xii).

Noble metal catalysts can be selected from platinum oxide, palladium on carbon and palladium hydroxide on carbon; preferably the noble metal catalyst is palladium on carbon and palladium hydroxide on carbon.

Polar solvents may be selected from methanol, ethanol, *n*-butanol; preferably ethanol.

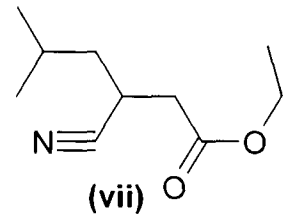
Generally, hydrogen pressure is maintained between 3 kg/cm$^2$ to 15 kg/cm$^2$; preferably 10 kg/cm$^2$.

Compound (xii) on reaction with thionyl chloride followed by ammonia gas purging gives compound (xiii) which is further converted to compound (vii) by thionyl chloride.

Dehydration of compound (xiii) to compound (vii) also may be carried out in presence of phosphorous pentoxide, phosphorous oxychloride.

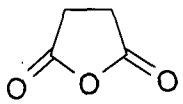
The above may be mentioned as under:

A process for synthesis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (vii)



**(vii)**

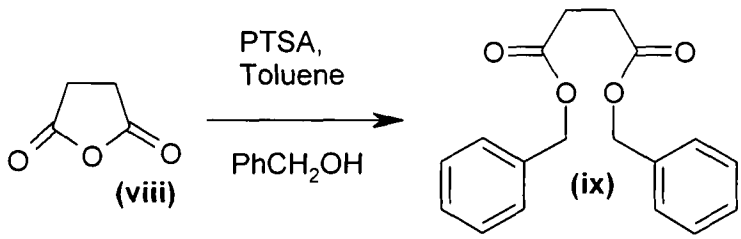
from dihydrofuran-2,5-dione of formula (viii)



**(viii)**

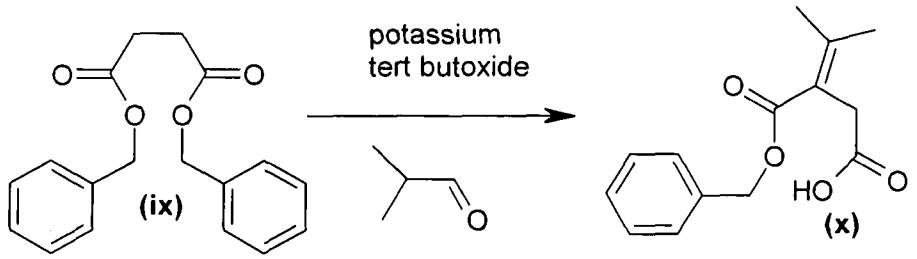
comprising,

a. reaction of dihydrofuran-2,5-dione of formula (viii) with benzylalcohol in presence of paratoluenesIfonic acid to give succinic acid dibenzyl ester (ix);

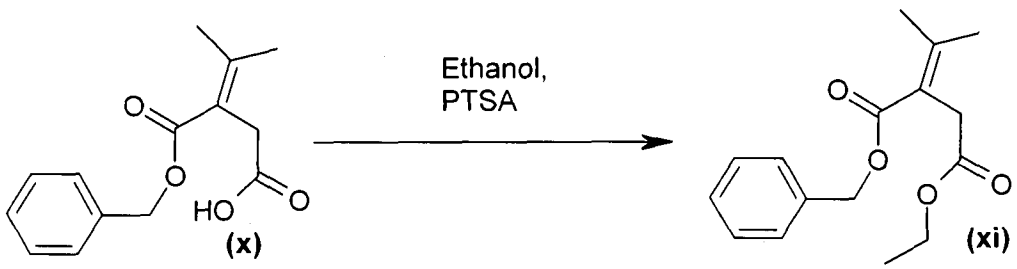



b. reaction of compound of formula (ix) with 2-Methyl-propionaldehyde in presence of a base selected from potassium *tert*-butoxide, sodium hydride, sodium ethoxide, sodium methoxide, preferably potassium *tert*-butoxide in a polar solvent selected from *tert*-butanol, tetrahydrofuran, dimethyl sulfoxide and dimethoxy ethane, preferably *tert*-butanol at temperature of about 25 to

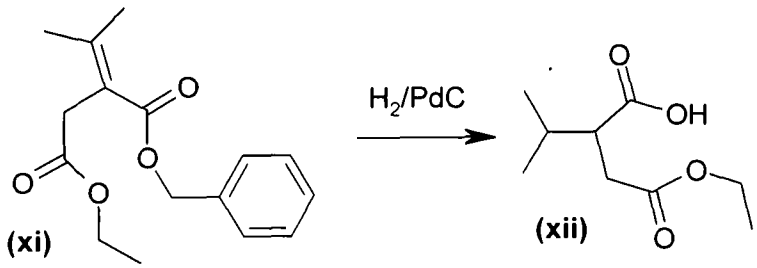
80 $^0$C, preferably at 50 to 60 $^0$C to give 2-isopropylidene-succinic acid 1-benzyl ester of formula (x);



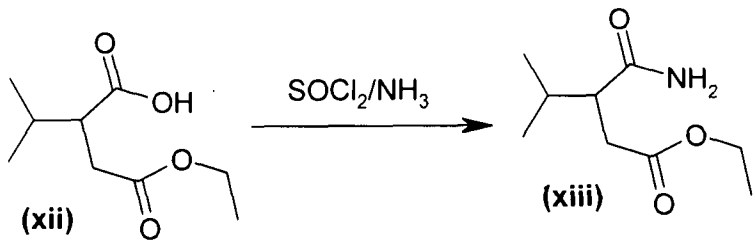

c. acid catalyzed esterification of compound of formula (x) with aliphatic alcohol selected from straight chain or branched, of $C_1$ to $C_6$ carbon chain length, preferably ethanol, wherein acid used is aryl sulfonic acid such as *para*-toluenesulfonic acid to get 2-isopropylidene-succinic acid 1-benzyl ester 4-ethyl ester (xi);



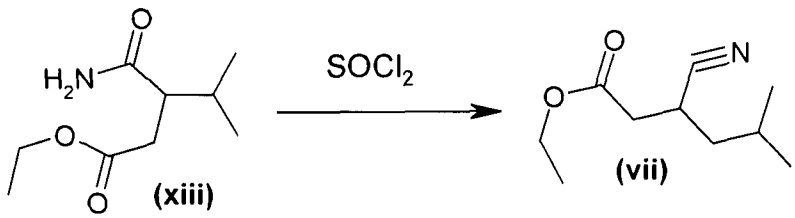

d. hydrogenation and hydrogenolysis of compound of formula (xi) in presence of noble metal catalyst selected from platinum oxide, palladium on carbon and palladium hydroxide on carbon; preferably the noble metal catalyst is palladium on carbon and palladium hydroxide on carbon in polar solvent such as methanol, ethanol, *n*-butanol, preferably ethanol at hydrogen pressure maintained between 3 kg/cm$^2$ to 15 kg/cm$^2$, preferably about 10 kg/cm$^2$ to get 2-isopropyl-succinic acid 4-ethyl ester (xii);




e. reaction of compound of formula (xii) with thionyl chloride to give acid chloride *in situ* treatment of acid chloride with ammonia to get 3-carbamoyl-5-methyl-hexanoic acid ethyl ester (xiii);




f. dehydration of compound of formula (xiii) in presence of dehydrating agent such as thionylchloride, phosphorous pentoxide, phosphorous oxychloride to get (*RS*)-3-cyano-5-methyl-hexanoic acid ethyl ester (vii);



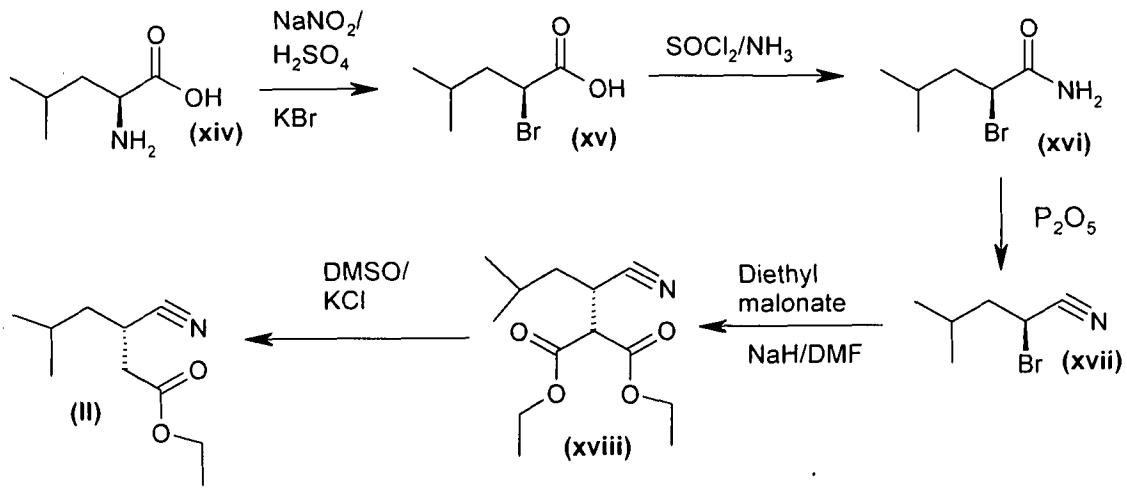

Such that at each step the intermediates were optionally isolated and purified with suitable process.

**B) The process for the preparation of enantiomerically enriched 3-cyano-5-methyl-hexanoic acid alkyl ester, preferably ethyl ester.**

**Route 3:** Scheme D outlines the reaction scheme for the preparation of enantiomerically enriched (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester (II) from *S*-leucine (xiv).

Compound (xiv) via a Sandmeyer reaction is converted to compound (xv) and thereafter the resulting compound (xv) is reacted with thionyl chloride and ammonia to obtain compound (xvi). Compound (xvi) is dehydrated in presence of phosphorus pentoxide to obtain compound (xvii) (Bulletin of the Chemical Society of Japan; vol. 43; (1970); p. 1443 – 1450)

Compound (xvii) on $S_N2$ displacement reaction with diethyl malonate yields enantiomerically enriched compound (xviii) which on further decarboxylation produces the enantiomerically pure compound (II).




**Scheme-D:** Route 3

Compound (xviii) is obtained by reacting compound (xvii) with diethyl malonate in presence of base in polar solvent.

Base used for synthesis of compound (xvii) is selected from sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate; preferably sodium hydride.

Polar solvent may be selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4 dioxane, and dimethoxy ethane; preferably dimethoxy ethane and N, N-dimethyl formamide; more preferably dimethoxy ethane.

Compound (xviii) is usually obtained by conducting reaction at temperature 10 to 80 $^0$C; preferably at 50 to 60 $^0$C.

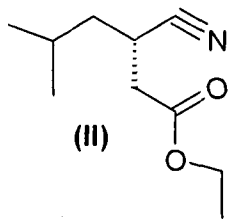
Compound (II) is obtained from compound (xviii) by decarboxylation performed in dimethyl sulfoxide with potassium chloride or sodium chloride; preferably potassium chloride.

Compound (II) is usually obtained by conducting reaction at temperature of about 140 to 180 $^0$C; preferably at 150 to 160 $^0$C.

It is worthwhile to note that, through employing different reaction conditions such as, different bases, solvents, and temperature, there is scope for improvement of the enantiomeric excess of compound (II) acquired from *S*-Leucine.

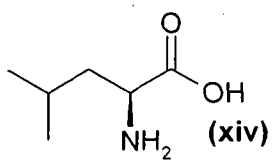
The above may be described as under:

A stereoselective process for synthesis of enantiomerically enriched (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II)




(II)

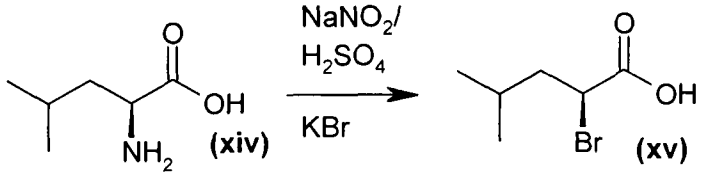
from *S*-leucine of formula (xiv)



(xiv)

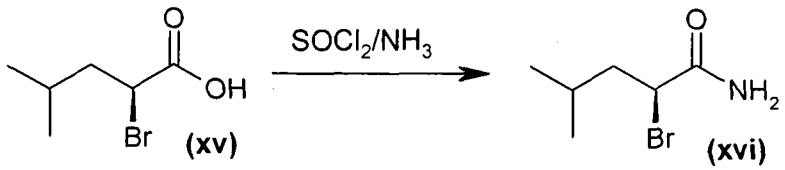
comprising;

(a) Samdmeyer reaction of (*S*)-2-amino-4-methyl-pentanoic acid of formula (xiv) using sodium nitrite and sulfuric acid followed by treatment of reaction mass with potassium bromide to get (*S*)-2-bromo-4-methyl-pentanoic acid (xv);



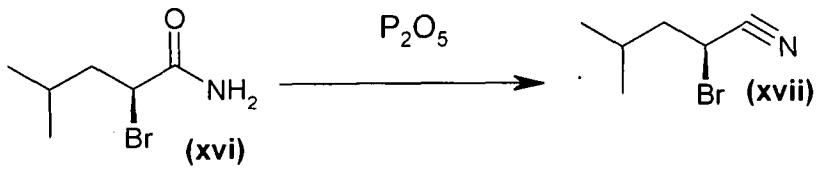
(xiv) (xv)

(b) reaction of compound of formula (xv) with thionyl chloride to get acid chloride, treatment of which ammonia *in situ* to get (*S*)-2-bromo-4-methyl-pentanoic acid amide (xvi);



(xv) (xvi)

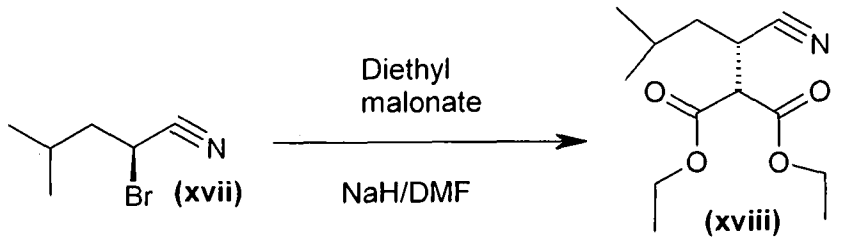
(c) dehydration of compound of formula (xvi) in presence of dehydrating agene such as phosphorous pentoxide to get (*S*)-2-bromo-4-methyl-pentanenitrile (xvii);



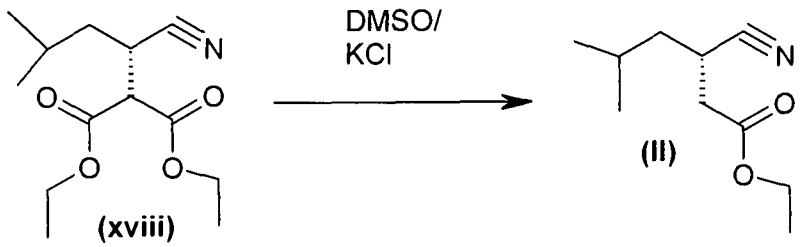
(xvi) (xvii)

(d) preparation of enantiomerically enriched 2-((*S*)-1-cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii) by $S_N2$ displacement reaction of compound of formula (xvii) with diethyl malonate in presence of base such as sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate,

preferably sodium hydride and polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4 dioxane, and dimethoxy ethane; preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably dimethoxy ethane at temperature of about 10°C to 80°C, preferably at 50°C to 60°C;




(e) decarboxylation of compound of formula (xviii) in presence of potassium chloride or sodium chloride in presence of solvent such dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get enantiomerically enriched (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester (II).

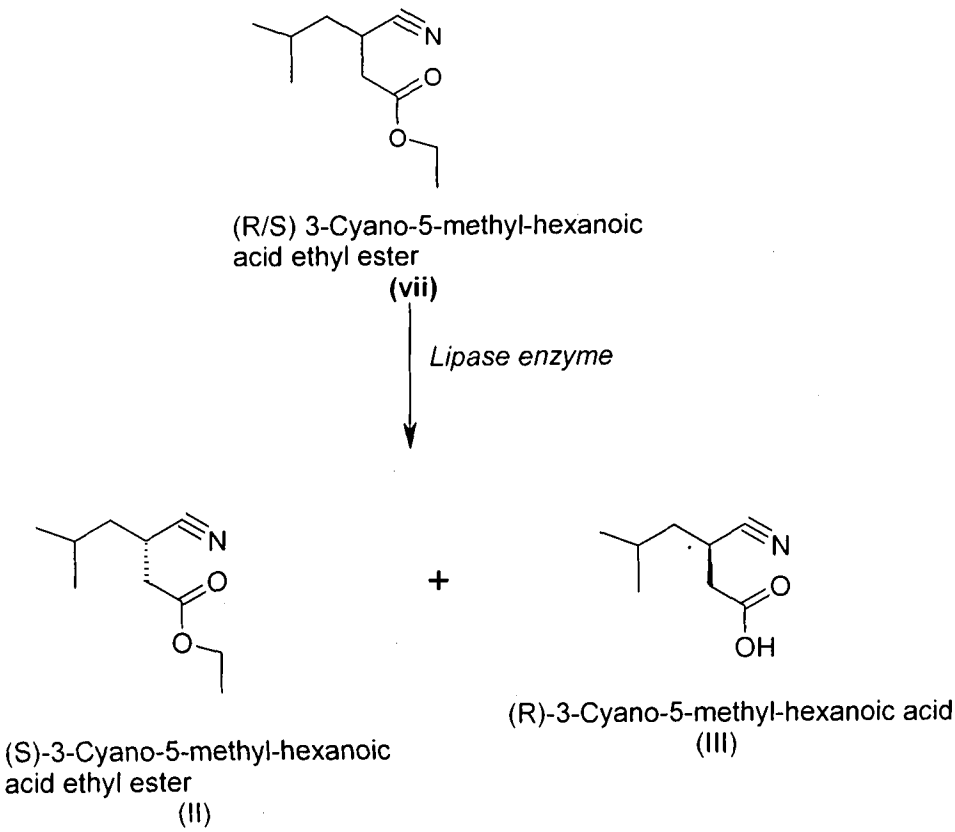



Such that at each step the intermediates were optionally isolated and purified with suitable process.

**C) Kinetic resolution of (*RS*)-3-cyano-5-methyl-hexanoic acid alkyl ester or enantiomerically enriched 3-cyano-5-methyl-hexanoic acid alkyl ester to obtain optically pure (*S*)- 3-cyano-5-methyl-hexanoic acid alkyl ester**

Stereo-selective enzymatic hydrolysis for resolution of ester is very well documented (Hydrolases in organic synthesis: Regio and stereo-selective biotransformation; Willey VCH publication.). However, it is difficult to predict enzymes and reaction

conditions, which would give desired stereo-selectivity in excellent yield as well as high optical purity.




**Scheme-E**

The present inventors have performed innovative experiments taking the following factors in consideration;

1) Selection of enzymes having desired stereo-selectivity as well as excellent rate of conversion towards the desired product.

2) Enzymes are very substrate specific and ratio of substrate concentration to enzyme is crucial for obtaining desired stereo-selectivity in good yield.

3) Enzymes are very sensitive to temperature. Hence, it is very essential to carry out reaction at optimized temperature for obtaining

maximum rate of reaction at highest stereo-selectivity and specificity for the substrate.

4) Enzymes are very sensitive to pH. Hence to obtain excellent yield and stereo-selectivity it is essential to optimize the pH.

Useful enzymes for stereo-selective hydrolysis of the compound (vii) include hydrolases, Screening of the different lipase is given in Table 1. Particularly useful lipases include enzymes derived from the microorganism *Candida antarctica B*.

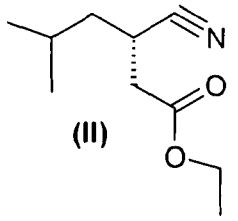
**Table 1: Screening of lipases for stereo-selective hydrolysis of (*RS*)-3-cyano-5-methylhexanoic acid ethyl ester or enantiomerically enriched (*S*)-3-cyano-5-methylhexanoic acid ethyl ester to (*S*)-3-cyano-5-methylhexanoic acid ethyl ester.**

| Enzyme | Trade Name | Supplier | % *ee* for S-ester |
|---|---|---|---|
| *Candida antarctica Lipase* B | Novozym 435 | Novozyme A/S | 99% |
| *Thermomyces langinous* | Lipozyme TL IM | Novozyme A/S | @ |
| *Rhizomucor miehei* | Lipozyme RM- IM | Novozyme A/S | @ |
| *Candida antarctica Lipase B* | CALB | C-LETA | 96% |
| *Candida antarctica Lipase B* | CALB-lyophilized | C-LETA | 99% |
| *Aspergillus niger* | Amano 'AS' | Amano enzyme Japan | @ |
| *Pseudomonas fluorescens* | Amano 'AK' | Amano enzyme Japan | @ |

| | | | |
|---|---|---|---|
| *Burkholderia cepacia* | Amano 'PS' IM | Amano enzyme Japan | @ |
| *Burkholderia cepacia* | Amano 'PS' SD | Amano enzyme Japan | @ |
| *Candida rugosa* | Amano 'AYS' | Amano enzyme Japan | @ |
| *Candida antarctica Lipase A* | CLEA | CLEA Techologies | 7% |
| *Rhizomucor miehei* | Rhizomucor miehei | Sigma | @ |
| *Thermomyces langinous* | *Thermomyces Langinous* | Sigma | @ |

@: No specificity observed.

Thus the present invention provide process for enantiomeric enrichment of (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II)



(II)

in its racemate or partially enriched form comprising stereoselective hydrolysis of *R* isomer of 3-cyano-5-methylhexanoic acid ethyl ester involving enzymatic kinetic resolution technique using lipase enzyme derived from the microorganism *Candida antarctica B* in presence of buffer at concentration from about 10 mM to 100mM, preferably 30 mM to 60 mM and more preferably 40 mM at temperature range from 10°C to 50°C, preferably at 15°C at pH ranging from 6 to 8, preferably at pH 7.2 and isolation of un-hydrolyzed (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II).

The rate of formation of (*R*) - 3-cyano-5-methylhexanoic acid and (*S*) - 3-cyano-5-methylhexanoic acid, is depicted in Figure 1 where the rate of formation of (*R*) - 3-cyano-5-methylhexanoic acid is 2.85 times more as compared to (*S*)-3-cyano-5-methylhexanoic acid at 25 $^0$C with 10% catalyst loading.

The rate of formation of (*R*)-3-cyano-5-methylhexanoic acid and (*S*) - 3-cyano-5-methylhexanoic acid is depicted in Figure 2 , where the rate of formation of (*R*)-3-cyano-5-methylhexanoic acid is 10 times more as compared to (*S*)-3-cyano-5-methylhexanoic acid at 15 $^0$C with 6% catalyst loading.

Initial concentration of compound (II) is in the range of 5% to 15 % (w/v) of the reaction volume and enzyme loading of about 5 % to 15 % (w/w) of the substrate (compound (II)); preferably 6% (w/w) of the substrate.

The stereo-selective hydrolyses are carried out at temperature range from 5 to 50 $^0$C, preferably at 15 $^0$C.

The stereo-selective hydrolysis is carried out at different pH ranges from 6 to 8, preferably at pH 7.2.

In the absence of pH control, the reaction mixture pH decreases as the hydrolysis of the substrate proceeds via the formation of carboxylic acid (compound III).

To compensate for this change, the hydrolysis reaction is carried out in presence of suitable buffer or pH is controlled externally though the addition of suitable base. Particularly, buffer used for reaction includes sodium phosphate, potassium phosphate, and sodium acetate; preferably sodium phosphate buffer is used.

The buffer concentration generally ranges from about 10 mM to 100mM; preferably 30 mM to 60mM and more preferably 40 mM.

A suitable base used for adjusting the buffer pH externally includes potassium hydroxide, sodium hydroxide and ammonium hydroxide.

(*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester obtained by enzymatic hydrolysis is converted into (*S*) - 3-cyano-5-methyl-hexanoic acid and specific optical rotation is measured. (J. Am. Chem. Soc., 2003, 125(15) 4442-43)

Optically pure (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester is converted into *S*-pregabalin by reported methods (US patent No. 5,637,767; J. Am. Chem. Soc., 2003, 125(15) 4442-43)

Recycling of undesired (*R*)-3-cyano-5-methylhexanoic acid is carried out through converting in corresponding ester, *i.e.* (*R*)-3-cyano-5-methylhexanoic acid ethyl ester.

(*R*)-3-cyano-5-methylhexanoic acid ethyl ester in alcoholic solvent in presence of base converted to (*RS*)-3-cyano-5-methylhexanoic acid ethyl ester.

Nomenclatures used for the compounds mentioned herein are as understood from the CambridgeSoft® ChemOffice software *ChemDraw Ultra version 6.0.1.*

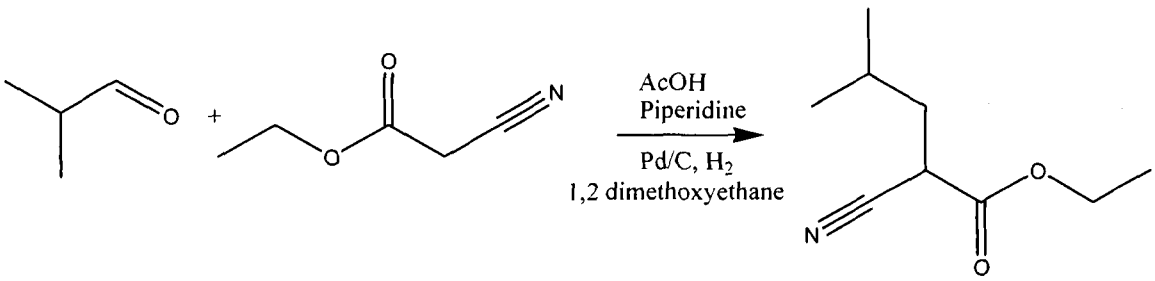
The enantiomeric excess (*ee*) for pregabalin is determined by HPLC using a Shimadzu LC 2010 system equipped with a chiral column (Purosphere star RP-18e (4.6 x 150mm), 5µm), column oven temperature 25 $^{0}$C and UV visible detector (UV at 340nm). Mobile phase is buffer: acetonitrile (55:45) with flow rate 1.0 $mL^{-1}$,

injection volume 20 µl. The enantiomeric excess (*ee*) is determined by derivatized by reacting with Marfey's reagent.

The enantiomeric excess (*ee*) for (*S*) - 3-cyano-5-methyl-hexanoic acid ethyl ester is determined by Gas-Liquid chromatography using a Shimadzu GC 2010 system equipped with a chiral column (Chiraledex (20m x 0.25mm x 0.12mm)), and FID detector.

NMR spectra are obtained at 200 and 400 MHz Bruker instruments, with $CDCl_3$ as solvent. Chemical shifts ($\delta$) are given in ppm relative to tetramethylsilane ($\delta$ = 0 ppm). IR spectra are recorded on Perkin Elmer Spectrum (Model: Spectrum 100) and absorption bands are given in $cm^{-1}$. Mass analyses are performed on Shimadzu LCMS 2010A instrument.

**Example 1:** Synthesis of 2-cyano-4-methyl-valeric acid ethyl ester (iv) from condensation of ethyl cyano acetate with *iso*-butyraldehdye.




Ethyl cyano acetate (56.5 g, 0.5 mol) was dissolved in dimethoxy ethane (100 mL) and *iso*-butyraldehyde (43.2 g, 0.6 mol) was added to it at room temperature. The mixture was cooled to 4 $^0$C and a solution of acetic acid (6 mL) and piperidine (2 mL) in 50 mL of dimethoxy ethane was added slowly over a period of 20 min by maintaining temperature below 20 $^0$C. The reaction mixture was transferred into a Parr autoclave reactor followed by addition of 2 % catalyst palladium on carbon (50 % wet (10% Pd loading)). Reactor was purged with hydrogen gas two times and charged with hydrogen,

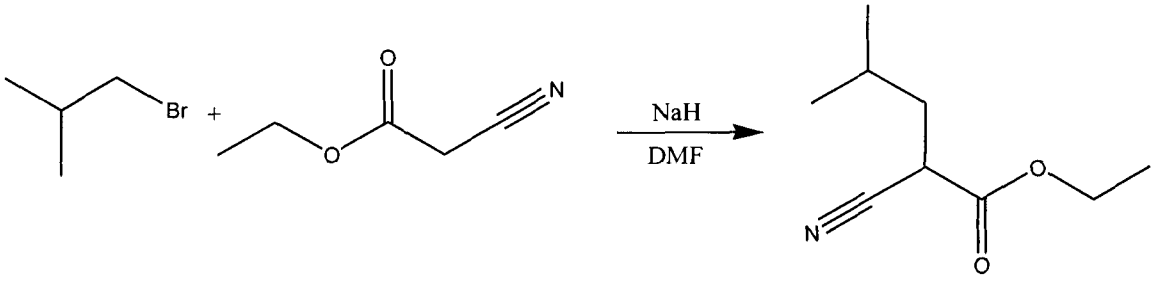
3 kg/cm$^2$ pressure was maintained in the Parr autoclave until hydrogen consumption ceases. Reaction was monitored by TLC. After completion of reaction, reaction mixture was filtered through Celite bed to remove Pd/C and filtrate was concentrated under reduced pressure to remove solvent. Residue was suspended in 100 mL water and extracted with di-*iso*-propyl ether (3 x 250 mL). After extraction, organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain 2-cyano-4-methyl-valeric acid ethyl ester (80 g, 95 % yield) as light yellow oil.

**FTIR (neat):** 2962, 2249, 1746, 1469, 1186 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.28 (t, 3H), 1.17-1.87 (m, 3H), 3.49 (q, 1H), 4.22 (q, 2H).

**MS (EI):** $C_9H_{15}NO_2$: 169.0; $[M+H_2O]^+$: 186.85 and $[M]^-$: 167.80

**Example 2:** Synthesis of 2-cyano-4-methyl-valeric acid ethyl ester (iv) from ethyl cyano acetate and *iso*-butyl bromide




A reactor was charged with 500 mL of dimethyl formamide and under nitrogen atmosphere sodium hydride (40.0 g, 1.0 mol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 – 15 $^0$C and a solution of ethyl cyano acetate (113.0 g, 1.0 mol) in 200 mL dimethyl formamide was added slowly over a period of 1 h by maintaining temperature below 20 $^0$C. The reaction mixture was heated to 50 $^0$C and stirred further for 1 h at 50 $^0$C. Solution of *iso*-butyl bromide (137.0

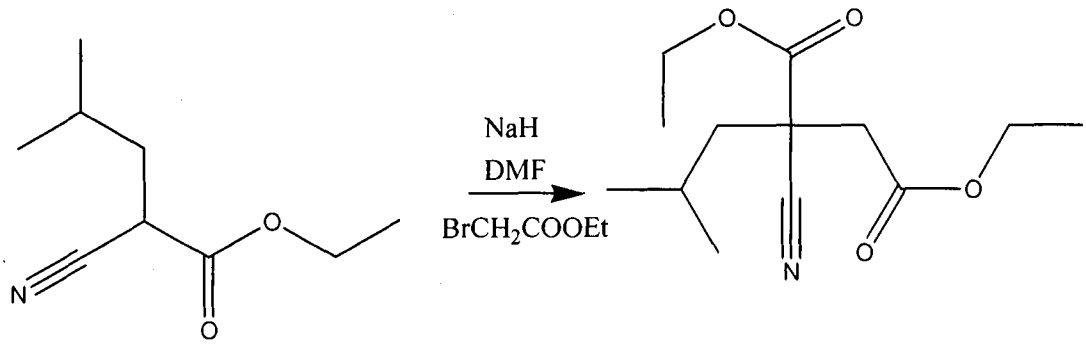
g, 1.0 mol) in 100 mL dimethyl formamide was added slowly to above reaction mixture over a period of 1 h. After complete addition of *iso*-butyl bromide solution, reaction mixture was cooled to room temperature and stirred additionally for 24 h. The reaction mixture was quenched by adding 1.0 L water. Aqueous layer was extracted with di-*iso*-propyl ether (3 x 500 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain crude product. Crude product was further purified through column chromatography using silica gel (100-200 mesh size) and mobile phase: [ethyl acetate: hexane] to obtain 2-cyano-4-methyl-valeric acid ethyl ester (66.8 g, 40 % yield) as light yellow oil.

**FTIR (neat):** 2962, 2249, 1746, 1469, 1186 $cm^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.28 (t, 3H), 1.17-1.87 (m, 3H), 3.49 (q, 1H), 4.22 (q, 2H).

**MS (EI):** $C_9H_{15}NO_2$: 169.0; $[M+H_2O]^+$: 186.85 and $[M]^-$: 167.80

**Example 3:** Synthesis of diethyl 2-cyano-2-isobutylsuccinate (vi) from compound (iv) and ethyl bromo acetate (v) in dimethyl formamide as solvent.




A reactor was charged with 50 mL of dimethyl formamide and under nitrogen atmosphere sodium hydride (2.83 g, 71.0 mmol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 to 15 $^0$C and solution of 2-cyano-4-methyl-valeric acid ethyl ester (iv) (10.0 g, 59.1 mmol) in 20 mL dimethyl formamide was added slowly over a period of 1 h by maintaining temperature below 20 $^0$C. The reaction

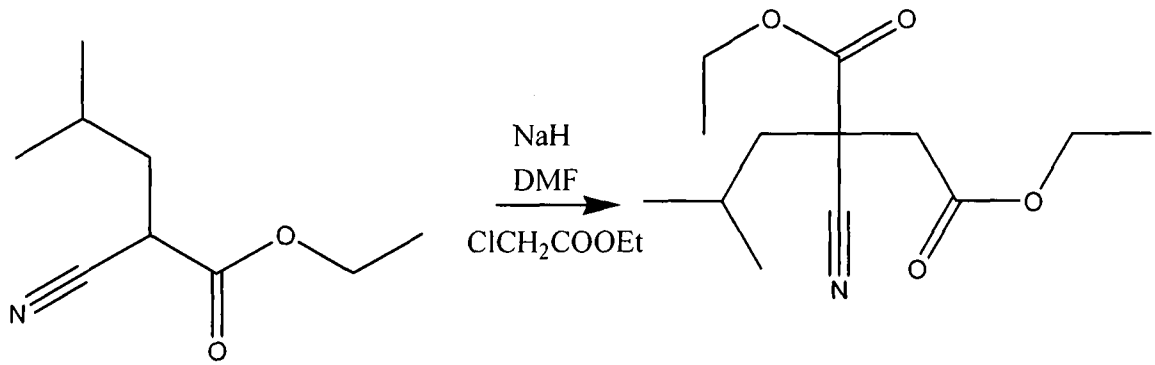
mixture was heated to 50 $^0$C and stirred further for 1 h at 50 $^0$C. A solution of ethyl bromoacetate (v) (11.84 g, 70.9 mmol) in 25 mL dimethyl formamide was added slowly to the reaction mixture over a period of 1 h. After complete addition of ethyl bromoacetate solution, reaction mixture was cooled to room temperature and stirred additionally for 24 h. The reaction mixture was quenched by adding 200 mL water. Aqueous layer was extracted with dichloromethane (3 x100 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain 2-cyano-2-isobutylsuccinate (vi) (12.5 g, 83% yield) as light brown oil.

**FTIR (neat):** 2963, 2248, 1743, 1469, 1195, 1025 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.23 (t, 3H), 1.28 (t, 3H), 1.70-1.89 (m, 3H), 2.80 (d, 1H), 3.02 (d, 1H), 4.16 (q, 2H), 4.28 (q, 2H).

**MS (EI):** $C_9H_{15}NO_2$: 255; $[M+H_2O]^+$: 273.05.

**Example 4:** Synthesis of diethyl 2-cyano-2-isobutylsuccinate (vi) from compound (iv) and ethyl chloro acetate (v) in dimethyl formamide as solvent.




A reactor was charged with 50 mL of dimethyl formamide and under nitrogen atmosphere sodium hydride (2.83 g, 71.0 mmol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 to15 $^0$C and a solution of 2-cyano-4-methyl-valeric acid ethyl ester (iv) (10 g, 59.1 mmol) in 20 mL dimethyl formamide was

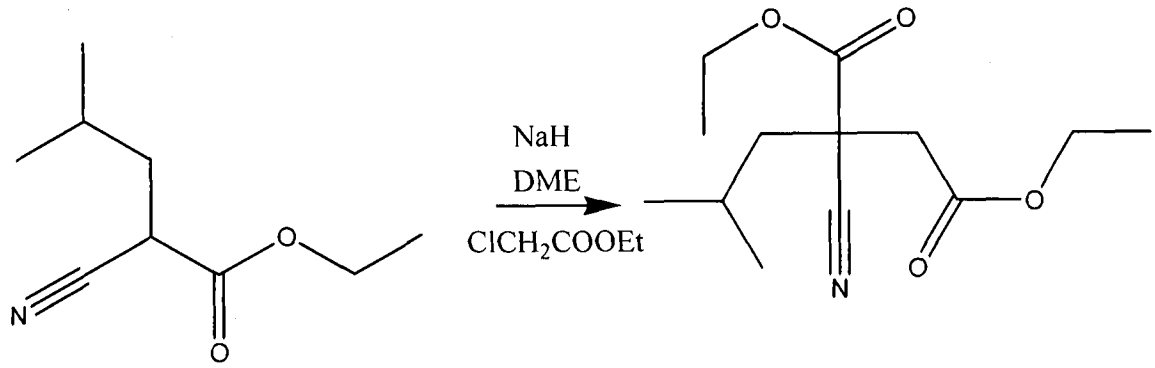
added slowly over a period of 1 h. by maintaining temperature below 20 $^0$C. The reaction mixture was heated to 50 $^0$C and stirred further for 1 h at 50 $^0$C. Solution of ethyl chloro-acetate (v) (9 g, 70.9 mmol) in 25 mL dimethyl formamide was added slowly to above reaction mixture over a period of 1 h. After complete addition of ethyl chloro-acetate solution, reaction mixture was cooled to room temperature and stirred additionally for 24 h.The reaction mixture was quenched by adding 200 mL water. Aqueous layer was extracted with 3x 100 mL dichloromethane. Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain 2-cyano-2-isobutylsuccinate (vi) (12.0 g, 80% yield).

**FTIR (neat):** 2963, 2248, 1743, 1469, 1195, 1025 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.23 (t, 3H), 1.28 (t, 3H), 1.70-1.89 (m, 3H), 2.80 (d, 1H), 3.02 (d, 1H), 4.16 (q, 2H), 4.28 (q, 2H).

**MS (EI):** $C_9H_{15}NO_2$: 255; $[M+H_2O]^+$: 273.05.

**Example 5:** Synthesis of diethyl 2-cyano-2-isobutylsuccinate (vi) from compound (iv) and chloro acetic acid ethyl ester (v) in dimethoxy ethane as solvent.




A reactor was charged with 50 mL of dimethoxy ethane and under nitrogen atmosphere sodium hydride (22.0 g, 550 mmol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 to 15 $^0$C and solution of 2-cyano-4-methyl-

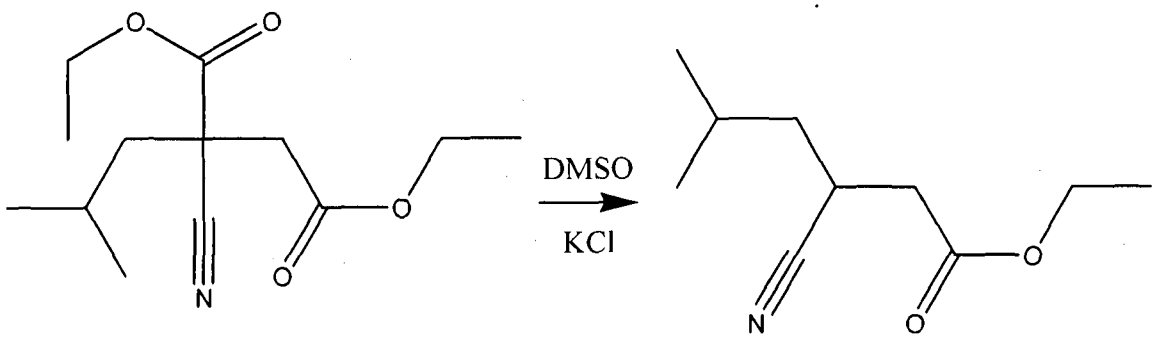
valeric acid ethyl ester (iv) (84.5 g, 0.5 mol) in 500 mL dimethoxy ethane was added slowly over a period of 1 h by maintaining temperature below 20 $^0$C. The reaction mixture was heated to 50 $^0$C and stirred further for 1 h at 50 $^0$C. Solution of ethyl chloroacetate (v) (74.0 g, 600 mmol) in 300 mL diethoxy methane was added slowly to above reaction mixture over a period of 1 h. After complete addition of ethyl chloroacetate solution, reaction mixture was cooled to room temperature and stirred additionally for 24 h. The mixture was filtered and filtrate was concentrated to remove solvent under reduced pressure to obtain 2-cyano-2-isobutylsuccinate (vi) (102 g, 80%).

**FTIR (neat):** 2963, 2248, 1743, 1469, 1195, 1025 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.23 (t, 3H), 1.28 (t, 3H), 1.70-1.89 (m, 3H), 2.80 (d, 1H), 3.02 (d, 1H), 4.16 (q, 2H), 4.28 (q, 2H).

**MS (EI):** $C_9H_{15}NO_2$: 255; $[M+H_2O]^+$: 273.05.

**Example 6:** Synthesis of (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester (vii)




A 50 mL reactor was charged with diethyl 2-cyano-2-isobutylsuccinate (vi) (102 g), potassium chloride (32.5 g) and dimethyl sulphoxide (500 mL). The resulting reaction mixture was heated at 150 to 160 $^0$C and maintained at that temperature for 4 h. Reaction was monitored by TLC for complete consumption of starting material. The reaction mixture was cooled to 40 to 50 $^0$C and treated with methyl *tert*-butyl ether (200 mL). The mixture was further cooled to 0 to 5$^0$C and treated with water (1 L) in small portions to maintain the temperature below 40 $^0$C. After stirring for 30 min the phases

were separated. The aqueous phase was extracted with methyl *tert*-butyl ether (3 x 800 mL), Organic phases were combined and washed twice with 100 mL water. The organic layer was decolorized by treating with 7.0 g of activated charcoal. The resultant mixture was filtered to remove charcoal and filtrate was evaporated to give (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester (vii) 76.1 g (98.5 % purity by GC) as light brown color oil.

**FTIR (neat):** 2961, 2242, 1738, 1469, 1182, 1023 $cm^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.22-1.24 (m, 4H), 1.58 (m, 1H), 1.83 (m, 1H), 2.49 (dd, 1H), 2.65 (dd, 1H), 2.98-3.06 (m, 1H), 4.17 (q, 2H). **$^{13}$C NMR ($CDCl_3$, 50 MHz):** 14.1, 21.2, 22.8, 25.8, 26.0, 37.1, 40.7, 61.4, 121.1, 169.7.

**MS (EI):** $C_{10}H_{17}NO_2$: 183; $[M+H_2O]^+$: 201.05.

**Example 7:** *One pot synthesis of (RS) - 3-cyano-5-methylhexanoic acid ethyl ester (vii)*

A reactor was charged with 50 mL of dimethoxy ethane and under nitrogen atmosphere sodium hydride (22.0 g, 550 mmol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 to15 $^0$C and solution of 2-cyano-4-methyl-valeric acid ethyl ester (iv) (80 g, 0.474 mol) in 500 mL dimethoxy ethane was added slowly over a period of 1 h by maintaining temperature below 20 $^0$C. The reaction mixture was heated to 50 $^0$C and stirred further for 1 h at 50 $^0$C. Solution of ethyl chloro-acetate (v) (74.0 g, 600 mmol) in 300 mL dimethoxy ethane was added slowly to reaction mixture over a period of 1 h. After complete addition of ethyl chloro acetate solution, reaction mixture was cooled to room temperature and stirred additionally for 24 h. Solvent was distilled under reduced pressure to obtain crude mixture of 2-cyano-2-isobutylsuccinate (vi) and sodium chloride. Dimethyl sulphoxide (500 mL) was added to crude mixture and reaction heated to 150$^0$ – 160 $^0$C and maintained at that temperature for 4 h. Reaction was monitored by TLC for complete consumption of starting material. The reaction mixture was cooled to 25 to 30 $^0$C and treated with methyl *tert*-butyl ether

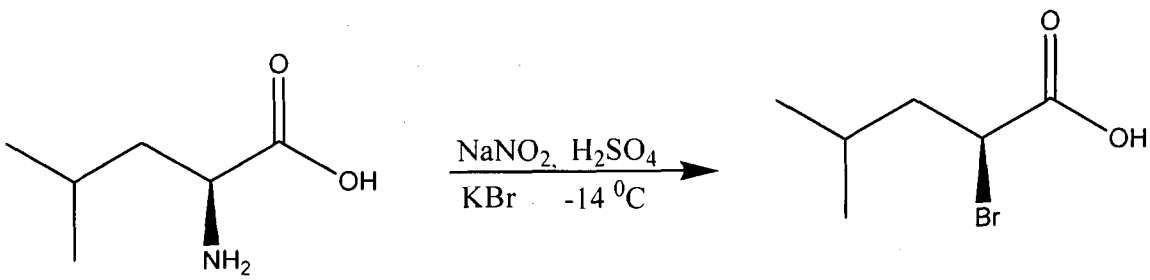
(200 mL). The mixture was further cooled to 0 to 5$^0$C and treated with water (1 L) in small portions to maintain temperature below 40 $^0$C. After stirring for 30 min the phases were separated. The aqueous phase was extracted with methyl *tert*-butyl ether (3 x 800mL). Organic phases were combined and washed with water. The organic layer was decolorized by treating with 7.0 g of activated charicoal (6.0 to 7.5 pH). The resultant mixture was filtered to remove charcoal and filtrate was evaporated to give (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester (vii) 76.1 g (98.5 % purity by GC).

**FTIR (neat):** 2961, 2242, 1738, 1469, 1182, 1023 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.22-1.24 (m, 4H), 1.58 (m, 1H), 1.83 (m, 1H), 2.49 (dd, 1H), 2.65 (dd, 1H), 2.98-3.06 (m, 1H), 4.17 (q, 2H). **$^{13}$C NMR ($CDCl_3$, 50 MHz):** 14.1, 21.2, 22.8, 25.8, 26.0, 37.1, 40.7, 61.4, 121.1, 169.7.

**MS (EI):** $C_{10}H_{17}NO_2$: 183; $[M+H_2O]^+$: 201.05.

**Example 8:** Synthesis of 2-(*S*)-bromo-4-methyl-pentanoic acid (xv) (Bulletin of the Chemical Society of Japan, 1970, 43, 1443 - 1450)




L-leucine (xiv) (86.0 g, 0.66 mol) was added to a solution of potassium bromide (2.72 g, 22.9 mmol) in sulphuric acid (162 g in 1.1.L of water) and the mixture was cooled to -14 $^0$C. Solution of sodium nitrite (70.0 g, 1.01 mol) in 200 ml water was added over period of 2 h and the mixture was stirred for 3 h at -14 $^0$C and subsequently for 1.5 h at 20 $^0$C. The reaction mixture was then extracted with dichloromethane (5 x 500 mL). Organic layer was separated and combined. The organic layer was dried over sodium sulphate

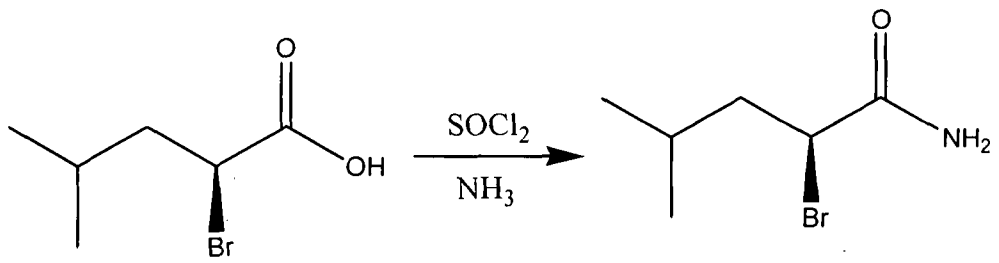
and concentrated under reduced pressure to obtain 2-(*S*)-bromo-4-methyl-pentanoic acid (xv) (108 g, 85% yield) as light yellow color oil.

**FTIR (neat):** 3583, 2959, 1718, 1468, 1258 $cm^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.72-1.86 (m, 1H), 1.93 (dd, 2H), 4.29 (t, 1H).

**MS (EI):** $C_6H_{11}BrO_2$: 193/195; $[M]^-$: 192.80/194.80.

**Example 9:** Synthesis of 2-(*S*)-bromo-4-methyl-pentanoic acid amide (xvi) (Bulletin of the Chemical Society of Japan, 1970, 43, 1443 - 1450)



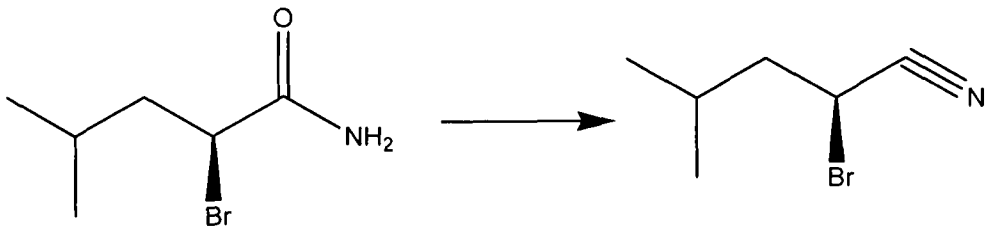

A reactor was charged with 2-(*S*)-bromo-4-methyl-pentanoic acid (xv) and 50 ml cyclohexane under nitrogen environment. The reaction mixture was heated to 60 $^0$C and thionyl chloride was added over a period of 1 h at 60 $^0$C. The mixture was further heated at 80°C for 12 h. The reaction mixture was cooled to 25 $^0$C and dichloromethane (1 L) was added. To this reaction mixture ammonia gas was purged for 1- 1.5 h. Reaction was monitored by TLC. After completion of reaction, ammonia solution (500 ml) was added. Organic layer was separated and aqueous layer was washed with 500 mL dichloromethane. Combined organic layer was dried organic layer over anhydrous sodium sulphate and solvent was removed under reduced pressure to obtain 2-(*S*)-bromo-4-methyl-pentanoic acid amide (xvi) (61.4 g, 77 % yield) as a white crystalline solid.

**FTIR (KBr):** 3363, 3188, 2957, 2871, 2364, 1666, 1419, 616 $cm^{-1}$.

**$^1$H NMR (DMSO-$D_6$, 200 MHz):** $\delta$ 0.83 (d, 3H), 0.88 (d, 3H), 1.54-81 (m, 3H), 4.35 (t, 1H), 7.26 (s, 1H), 7.76 (s, 1H).

**MS (EI):** $C_6H_{12}BrNO$: 192/194; $[M]^+$: 193.85/195.75.

**Example 10:** Preparation of 2-(*S*)-Bromo-4-methyl-pentanenitrile (xvii) (Bulletin of the Chemical Society of Japan, 1970, 43, 1443 - 1450)



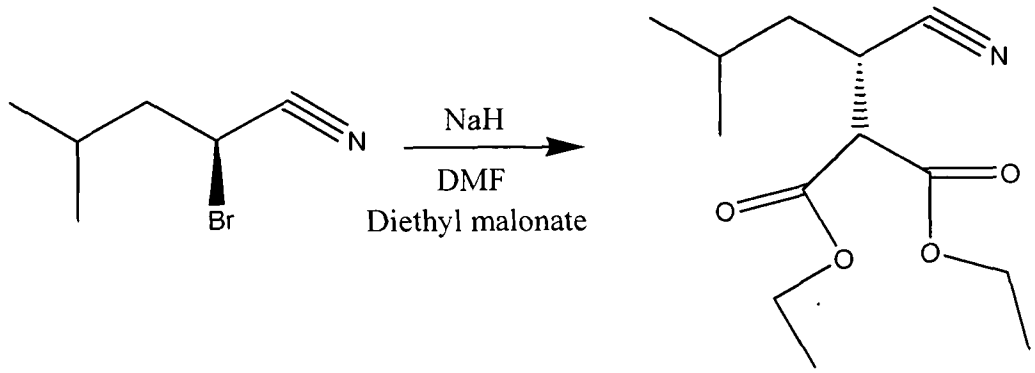

2-(*S*)-bromo-4-methyl-pentanoic acid amide (xvi) (50 g, 257.7 mmol) and phosphorous pentoxide (80.0 g) was mixed thoroughly and kept for vacuum distillation at 80 $^0$C and 12 mm of Hg to obtain 2-(*S*)-Bromo-4-methyl-pentanenitrile (xvii) 43 g (94 %) as a colorless oil.

**FTIR (Neat):** 2963, 2936, 2248, 1756, 1470, 1372, 746 $cm^{-1}$.

**$^1$H NMR (DMSO-$D_6$, 200 MHz):** $\delta$ 0.97 (d, 3H), 1.01 (d, 3H), 1.88-1.97 (m, 3H), 4.44 (t, 1H).

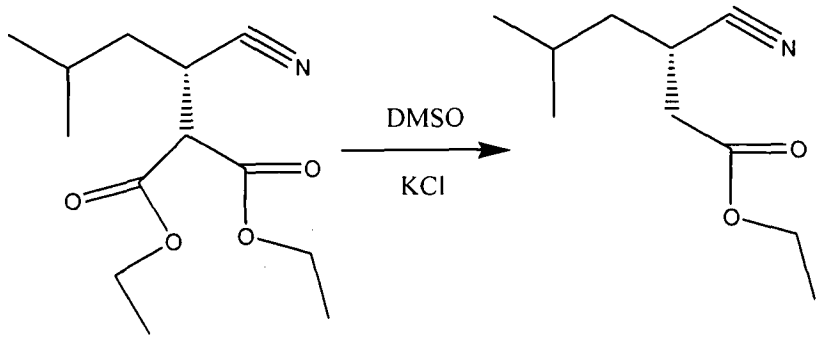
**MS (EI):** $C_6H_{10}BrN$: 175/177; $[M]^+$: 175.85.

**Example 11:** Preparation of 2-(1-Cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii)




A reactor was charged with 200 mL of dimethyl formamide and under nitrogen atmosphere sodium hydride (5.76 g, 144.3 mmol: 60% emulsion in paraffin) was added in small portions. The mixture was cooled to 10 – 15 $^0$C and solution of diethyl malonate ( 23.1 g, 0.145 mol) in 50 mL dimethyl formamide was added slowly over a period of 0.5 h. by maintaining temperature below 15 $^0$C. The reaction mixture heated and stirred at 25 $^0$C for 1 h. Solution of 2-bromo-4-methyl-pentanenitrile (xvii) (28 g, 144.3 mmol) in 50 mL dimethyl formamide was added slowly to above reaction mixture over a period of 0.5 h and stirred additionally for 24 h. The reaction mixture was quenched by adding 1 L water. Aqueous layer was extracted with (3 x500 mL) dichloromethane. Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain 2-(1-cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii) (40g).

**Example 12:** Synthesis of enantiomerically enriched (*S*)-3-cyano-5-methylhexanoic acid ethyl ester (II) (S:R; 78:22).



A 500 mL reactor was charged with 2-(1-cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii) (30 g), potassium chloride (9.65 g), water (10 mL) and dimethyl sulphoxide (300 mL). The resulting reaction mixture was heated at 150 to 160 $^0$C and maintained at that temperature for 4 h. Reaction was monitored by TLC for complete consumption of starting material. The reaction mixture was cooled to 40 to 50 $^0$C and treated with methyl *tert*-butyl ether (200 mL). The mixture was further cooled to 0 to 5 $^0$C and treated with water (1 L) in small portions to maintain temperature below 40 $^0$C. After stirring for 30 min the phases were separated. The aqueous phase was extracted with methyl *tert*-butyl ether (3x 800mL), Organic phases were combined and washed with water. The water phase was discarded. The organic layer was decolorized by treating with 7.0 g of activated charcoal. The resultant mixture was filtered and filtrate was evaporated to give enantiomerically enriched (*S*) - 3-cyano-5-methylhexanoic acid ethyl ester (II) (78:22, *S: R*) (17.5 g)

**FTIR (neat):** 2961, 2242, 1738, 1469, 1182, 1023 cm$^{-1}$.
**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 0.95 (d, 3H), 0.96 (d, 3H), 1.22-1.24 (m, 4H), 1.58 (m, 1H), 1.83 (m, 1H), 2.49 (dd, 1H), 2.65 (dd, 1H), 2.98-3.06 (m, 1H), 4.17 (q, 2H). **$^{13}$C NMR ($CDCl_3$, 50 MHz):** 14.1, 21.2, 22.8, 25.8, 26.0, 37.1, 40.7, 61.4, 121.1, 169.7.
**MS (EI):** $C_{10}H_{17}NO_2$: 183; $[M+H_2O]^+$: 201.05.

**Example 13:** Screen of enzymes for stereo-selective hydrolysis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester

Enzyme screening was carried out using HLC Heating-ThermoMixer (Model No. MHR 11) having 14 vial (14 x 10 mL) chamber blocks with orbital shaking. Each 10 ml vial contains 5 ml of phosphate buffer (pH 7.2), (RS) 3-cyano-5-methylhexanoic acid ethyl ester (0.5 g) and different enzyme as mentioned in Table 1 (50 mg). The reaction mixture was stirred for 4 h. Samples were withdrawn at defined interval and extracted

with dichloromethane and monitored on chiral GC analysis for stereo-selectivity of enzymes.

**Example 14:** Stereo-selective hydrolysis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester in presence of Novozym 435 at 25 $^0$C

A reactor equipped with overheard stirring was charged with 500 mL of sodium phosphate buffer, (40 mM, pH 7.2) and (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester (55.5 g) at 25 $^0$C. The mixture was stirred for 5 min and pH was adjusted to pH 7.2 with 1M solution of sodium hydroxide. Novozym 435 (5.5 g, 10% w/w of substrate) was added and the resulting reaction mixture was titrated with 1 M solution of sodium hydroxide to maintain a pH of 7.2. The extent of reaction was monitored on GC for chiral purity. After complete hydrolysis of (*R*) 3-cyano-5-methylhexanoic acid ethyl ester to corresponding acid, reaction was stopped by filtering the enzyme. Aqueous layer was extracted with dichloromethane (3 x100 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain (*S*) 3-cyano-5-methylhexanoic acid ethyl ester, (6.5 g, 25% yield, 98% *ee*).

**Example 15:** Stereo-selective hydrolysis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester in presence of Novozym 435 at 15 $^0$C

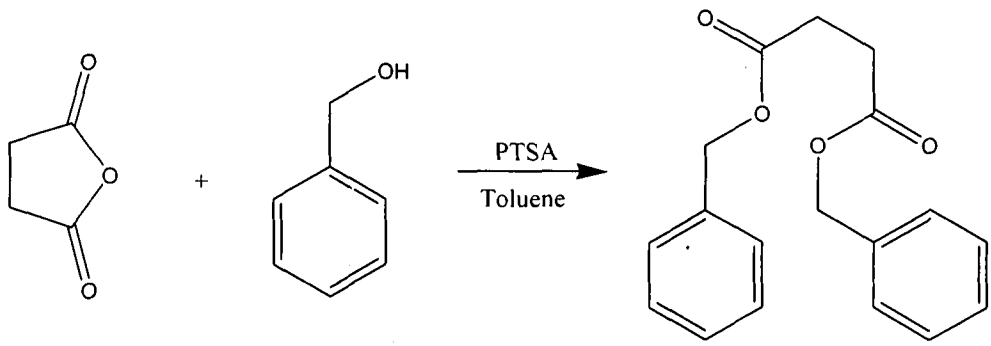
A reactor equipped with overheard stirring was charged with 500 mL of sodium phosphate buffer, (40 mM, pH 7.2) and (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester (7.0 g) at 15 $^0$C. The mixture was stirred for 5 min and pH was adjusted to pH 7.2 with 1M solution of sodium hydroxide. Novozym 435 (0.42 g, 6 % w/w of substrate) was added and the resulting reaction mixture was titrated with 1 M solution of sodium hydroxide to maintain a pH of 7.2. The extent of reaction was monitored on GC for chiral purity. After complete hydrolysis of (*R*) 3-cyano-5-methylhexanoic acid ethyl ester to

corresponding acid, reaction was stopped by filtering the enzyme. Aqueous layer was extracted with dichloromethane (3 x100 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain (*S*) 3-cyano-5-methylhexanoic acid ethyl ester (2.2 g, 62 % yield, 98% *ee*).

**Example 16:** Stereo-selective hydrolysis of enantiomerically enriched (*S*)-3-cyano-5-methylhexanoic acid ethyl ester (S:R; 78:22) in presence of Novozym 435 at 15 $^0$C

A reactor equipped with overheard stirring was charged with 250 mL of sodium phosphate buffer, (40 mM, pH 7.2) and enantiomerically enriched (*S*) - 3-cyano-5-methylhexanoic acid ethyl ester (6.0 g) at 15 $^0$C. The mixture was stirred for 5 min and pH was adjusted to pH 7.2 with 1M solution of sodium hydroxide. Novozym 435 (0.360 g, 6 % w/w of substrate) was added and the resulting reaction mixture was titrated with 1 M solution of sodium hydroxide to maintain a pH of 7.2. The extent of reaction was monitored on GC for chiral purity. After complete hydrolysis of (*R*) 3-cyano-5-methylhexanoic acid ethyl ester to corresponding acid, reaction was stopped by filtering the enzyme. Aqueous layer was extracted with dichloromethane (3 x100 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain (*S*) 3-cyano-5-methylhexanoic acid ethyl ester (3.3 g, 77 % yield, 99 % *ee*).

**Example 17:** Synthesis of dibenzyl succinate (ix)



A reactor equipped with overheard stirring and Dean Stark condenser was charged with 600 mL of toluene, succinic anhydride (100 g, 1 mol), *p*-toluene sulphonic acid (10 g, 10%w/w of succinic anhydride) and benzyl alcohol (108g, 1mol) at 25 $^0$C. The mixture heated to 100 $^0$C for 1 h. One more mole of the benzyl alcohol (108 g, 1 mol) was charged by over a period of 2 h and stirred further for 3 h at 135 $^0$C.

Reaction was monitored from the equivalent amount of water was collected in Dean-Stark condenser. After completion of reaction, toluene was removed by distillation and reaction mixture was cooled to room temperature. Residue was extracted with ethyl acetate (500 mL) and organic layer was washed 10 % solution of sodium bicarbonate (250 ml) to remove impurities.

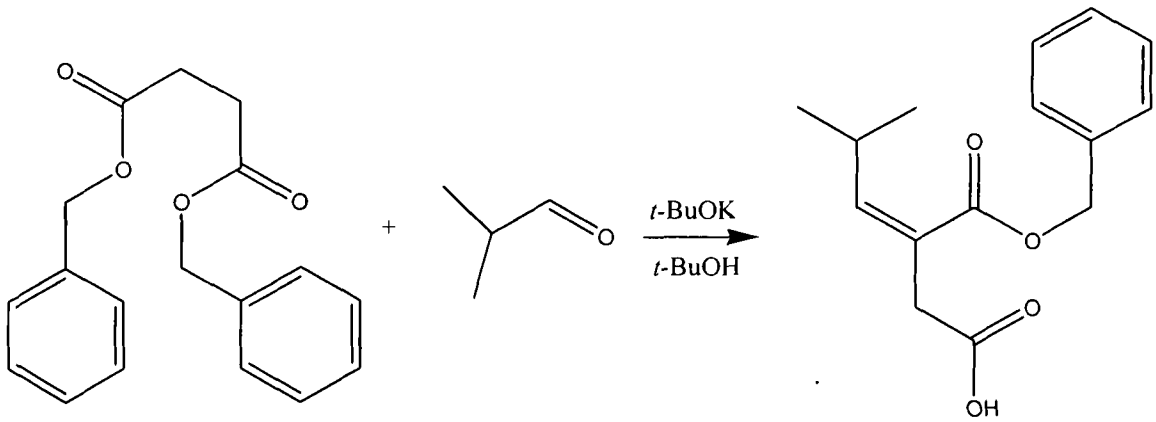
Organic layer was dried over anhydrous sodium sulphate and concentrated under reduced vacuum to obtain crude product, which was further purified through crystallization in hexane (300 mL) to obtain dibenzyl succinate (258 g) as light yellow crystalline material (ix).

**FTIR (Neat):** 3088, 3031, 1732, 1498, 1156, 1003, 733, 698 cm$^{-1}$.

**$^1$H NMR (DMSO-D$_6$, 200 MHz):** $\delta$ 2.73 (s, 4H), 5.16 (s, 4H), 7.38 (s, 10H).

**MS (EI):** $C_{18}H_{18}O_4$: 298; $[M^+ + H]^+$: 299.05.

**Example 18:** Synthesis of 3-((benzyloxy)carbonyl)-5-methylhex-3-enoic acid (x)

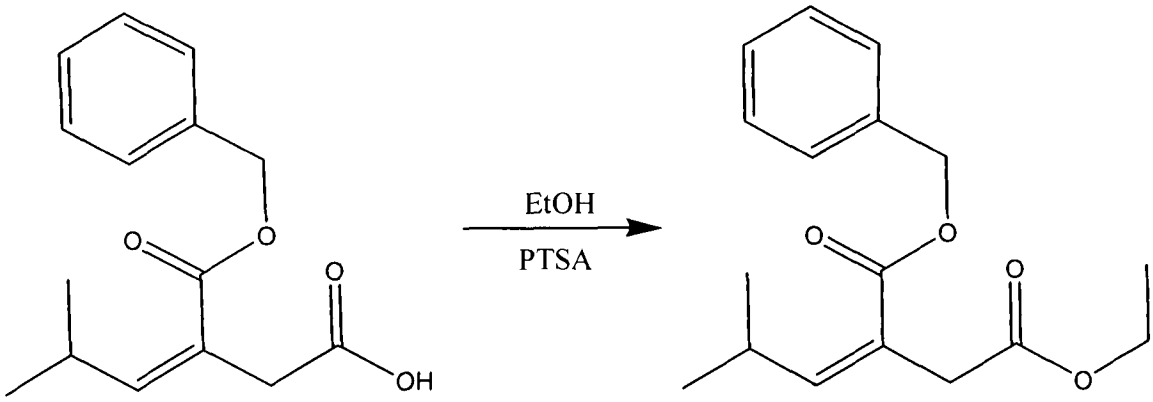



A reactor equipped with overheard stirring was charged with 250 mL *tert*- butanol and potassium *tert*-butoxide (82.7 g, 0.74 mol) at 25 $^0$C. Reaction mixture was heated to 50 $^0$C and solution of dibenzyl succinate (ix) (200 g, 0.67 mol) and iso-butyraldehyde (48.3 g, 0.67 mol) in 100 mL *tert*-butnaol was added in reaction mixture over period of 1 h by maintaining temperature 50 -55 $^0$C. After complete addition reaction mixture was stirred for 2 h at 70 $^0$C and further stirred for 12 h at 25 $^0$C. After completion of reaction solvent was removed under reduced pressure and residue was dissolved in water. Aqueous layer was extracted with ethyl acetate to remove un-reacted dibenzyl succinate.

Aqueous layer was acidified with hydrochloric acid (6 M, 200 mL) and extracted with ethyl acetate (3 x 100 mL). Combined organic layer was dried over anhydrous sodium sulfate and solvent was removed under reduced pressure to obtain crude product (x) (130.4 g, 74.5 % yield) as light brown oil.

**FTIR (Neat):** 3500, 2964, 1735, 1708, 1948, 1497, 1379, 1268, 1076, 991 cm$^{-1}$.
**MS (EI):** $C_{15}H_{18}O_4$: 262; $[M]^+$: 262.90.

**Example 19:** Synthesis of 3-((benzyloxy)carbonyl)-5-methylhex-3-enoic acid ethyl ester (xi)



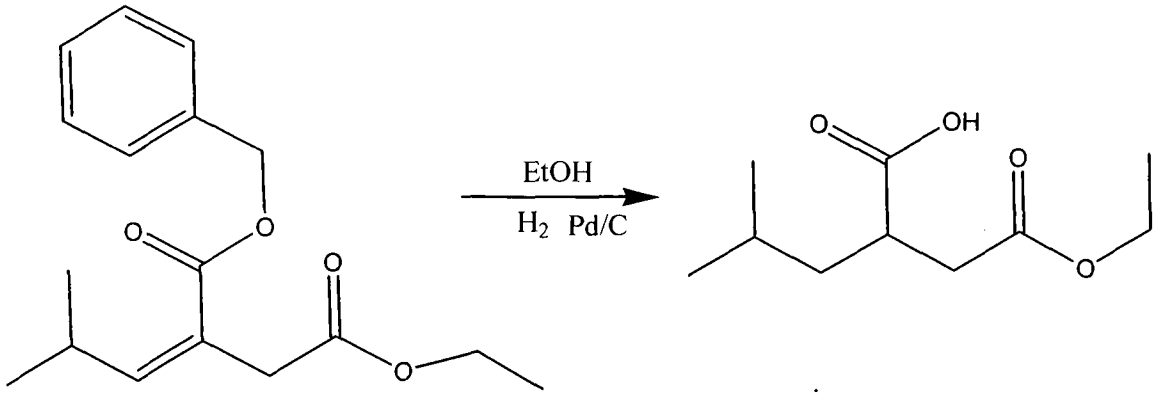

A reactor equipped with overheard stirring was charged with 500 mL ethanol,3-((benzyloxy)carbonyl)-5-methylhex-3-enoic acid (x) (78 g, 0.29 mol ) and *p*-toluene sulfonic acid (7.8g, 10% w/w of substrate) at 25 $^0$C. The mixture was heated to 90 $^0$C and stirred for 12 h at 90 $^0$C. Reaction was monitored in TLC. After completion of reaction excess of ethanol was removed by distillation under reduce pressure. Residue was dissolved in 5 % sodium carbonate (120 mL) and extracted with di-*iso*-propyl ether (3 x100 mL). Combined organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain product 3-((benzyloxy) carbonyl)-5-methylhex-3-enoic acid ethyl ester (xi) (61.8g, 72 % yield) as yellow oil.

**FTIR (Neat):** 3065, 3033, 2962, 1736, 1711, 1649, 1498, 1264, 1171, 1149, 1073, 993, 771, 697 cm$^{-1}$.

**$^1$H NMR ($CDCl_3$, 200 MHz):** $\delta$ 1.02 (d, 3H), 1.03 (d, 3H), 1.20 (t, 3H), 2.58 (m, 1H), 3.37 (s, 2H), 4.10 (t, 2H), 4.5.13 (s, 2H), 6.85(d, 1H), 7.33-7.34 (m, 5H).

**MS (EI):** $C_{17}H_{22}O_4$: 290; $[M]^+$: 290.85.

**Example 20:** Synthesis of 2-((ethoxycarbonyl) methyl)-4-methylpentanoic acid (xii)




A Parr autoclave reactor was charges with 3-((benzyloxy) carbonyl)-5-methylhex-3-enoic acid ethyl ester (xi) (43 g, 0.15 mol ) and ethanol 100 mL followed by addition of 10 % (% w/w of substrate) palladium on carbon (50 % wet (10% Pd loading)). Reactor was purged with hydrogen gas two times and charged with hydrogen, 10 $kg/cm^2$ pressure was maintained in the Parr autoclave until hydrogen consumption ceases. Reaction was monitored by TLC. After completion of reaction, reaction mixture was filtered through Celite bed to remove Pd/C and filtrate was concentrated under reduced pressure to remove solvent. Residue was dissolved in 1 M sodium hydroxide solution (150 mL). Aqueous layer was extracted with ethyl acetate to remove un-reacted material.

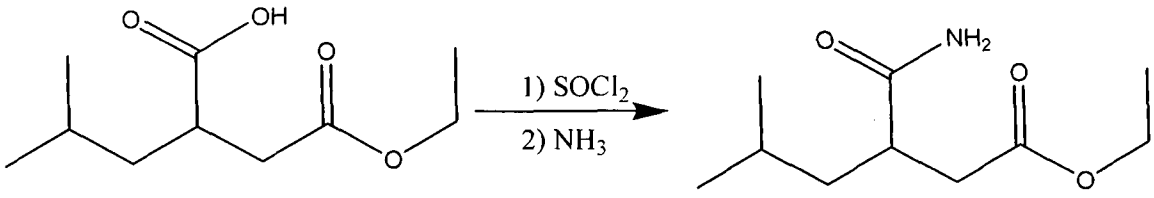
Aqueous layer was acidified with aqueous solution of hydrochloric acid (50%, 30 ml) and extracted with di-*iso*-propyl ether (3 x 250 mL). After extraction, organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain 2-((ethoxycarbonyl) methyl)-4-methylpentanoic acid (xii) (23 g, 76.9 % yield) as a light yellow oil.

**FTIR (Neat):** 3451, 2959, 2872, 1735, 1468, 1176, 1033 $cm^{-1}$.

**$^1$H NMR (DMSO-$D_6$, 200 MHz):** $\delta$ 0.82 (d, 3H), 0.86 (d, 3H), 0.98-1.22 (m, 4H), 1.35-1.59(m, 2H), 2.46 (t, 2H), 2.57-2.71(m, 1H), 4.01(t, 2H).

**MS (EI):** $C_{10}H_{18}O_4$.: 202.25; $[M]^+$: 202.90.

**Example 21:** Synthesis of ethyl 3-carbamoyl-5-methylhexanoate (xiii)



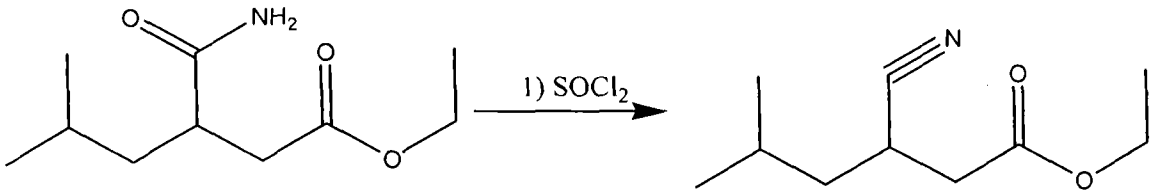

A reactor was charged with 2-((ethoxycarbonyl)methyl)-4-methylpentanoic acid (xii) (21 g, 0.1 mol) and 50 ml cyclohexane under nitrogen environment. The reaction mixture was heated to 60 $^0$C and thionyl chloride (18.6 g, 0.15 mol) was added over a period of 1 h at 60 $^0$C. The mixture was further heated at 80°C for 12 h. The reaction mixture was cooled to 25 $^0$C and dichloromethane (1 L) was added. To this reaction mixture ammonia gas was purged for 1- 1.5 h. Reaction was monitored on by TLC. After completion of reaction, ammonia solution (500 ml) was added. Organic layer was separated and aqueous layer was washed with 500 mL dichloromethane. Combined organic layer was dried organic layer over anhydrous sodium sulphate and solvent was removed under reduced pressure to obtain ethyl 3-carbamoyl-5-methylhexanoate (xiii) (18.0 g, 86 % yield) as yellow oil.

**FTIR (Neat):** 3428, 3354, 2958, 2873, 1733, 1674, 1468, 1414, 1373, 11791034 787 $cm^{-1}$.

**$^1$H NMR (DMSO-$D_6$, 200 MHz):** $\delta$ 0.88 (dd, 6H), 1.12-1.24 (m, 4H), 1.51-1.60 (m, 2H), 2.33 (dd, 1H), 2.56-2.78 (m, 2H), 4.05(t, 2H), 6.08 (s, 1H), 6.17 (s, 1H).

**MS (EI):** $C_{10}H_{19}NO_3$: 201; $[M]^+$: 201.95.

**Example 22:** Synthesis of (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester (vii)




A reactor equipped with overheard stirring was charged ethyl 3-carbamoyl-5-methylhexanoate (xiii) (15.1g, 74.6 mmol). The reaction mixture was heated at 80 $^0$C and thionyl chloride (10 mL, 137.4 mmol) was added to reaction mixture over period of 1 h. The reaction mixture was further stirred for 12 h at 80 $^0$C and then cooled to room temperature. Reaction mixture was quenched by adding water by maintaining reaction temperature below 25 $^0$C. The aqueous layer was extracted with di *iso*-propyl ether (2 x 50 ml). Combine organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain crude (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester (vii) (11 g, 80.5 % yield). Product was analyzed on gas liquid chromatography.

**Example 23:** Synthesis of (*RS*)-Pregabalin

The compound [III] obtained in the example was readily converted to pregabalin by reference of a prior literature (US patent No. 5,637,767; J. Am. Chem. Soc., 2003, 125(15) 4442-43)

**Example 24:** Synthesis of (*S*)-Pregabalin

The compound [II] obtained in the example was readily converted to pregabalin by reference of a prior literature (US patent No. 5,637,767; J. Am. Chem. Soc., 2003, 125(15) 4442-43)

**Example 25:** Preparation of ethyl ester of enantiomerically enriched (*R*) 3-cyano-5-methylhexanoic acid.

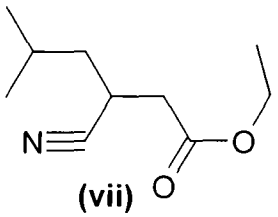
A reactor equipped with overheard stirring was charged with 50 mL of dichloromethane 50 mL, ethanol (1.24 g), (*R*) - 3-cyano-5-methylhexanoic acid (2.1 g) and DCC (5.58 g) at 0 $^0$C. The mixture was stirred for 1 h at 0 $^0$C. Further it was stirred for 12 h at 25 $^0$C. The extent of reaction was monitored on GC for chiral purity for (*R*) 3-cyano-5-methylhexanoic acid ethyl ester (1.8 g)

**Example 26:** Racemization of enantiomerically enriched (*R*) 3-cyano-5-methylhexanoic acid ethyl ester to (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester.

A reactor equipped with overheard stirring was charged with 50 ml ethanol, (*R*) - 3-cyano-5-methylhexanoic acid ethyl ester (2.4g) and sodium ethoxide (0.25 g) at 25 $^0$C. The mixture was stirred at 85 $^0$C for 6 h. The extent of reaction was monitored on GC for chiral analysis. After complete racemization of (*R*) - 3-cyano-5-methylhexanoic acid ethyl ester to (*RS*) - 3-cyano-5-methylhexanoic acid ethyl ester, solvent was removed under reduced pressure and water was added to the residue. Aqueous layer was extracted with dichloromethane (3 x100 mL). Organic layer was separated and combined. Combined organic layer was dried over anhydrous sodium sulphate and solvent was evaporated under reduced pressure to obtain (*RS*) 3-cyano-5-methylhexanoic acid ethyl ester (2.0 g).

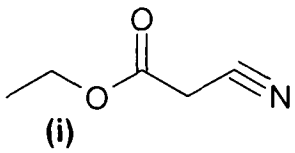
## CLAIMS

1. A process for synthesis of 3-cyano-5-methylhexanoic acid ethyl ester of formula (vii)



(vii)

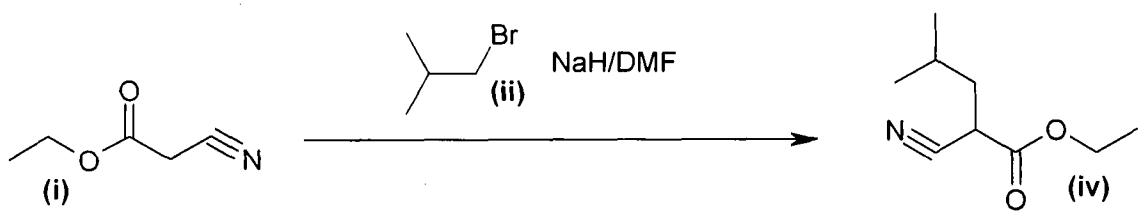
from ethyl cyano acetate of formula (i)



(i)

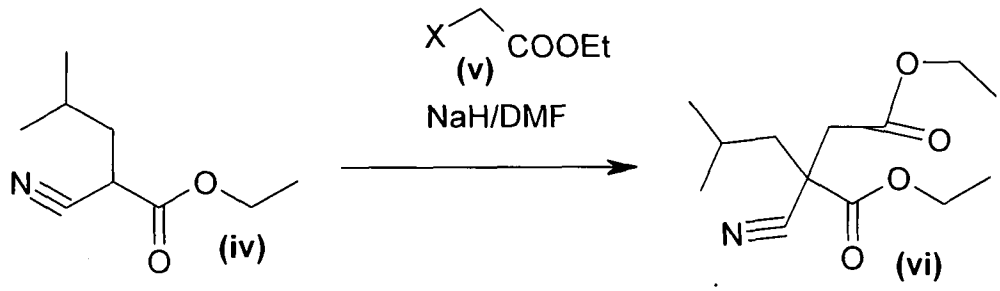
comprising,

a) condensation of cyanoacetic acid ethyl ester with 1-bromo-2-methyl propane in presence of base such as alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride in a polar solvent such as 1,4-dioxane, tetrahydrofuran, dimethoxy ethane, and diglyme, preferably dimethoxy ethane and diglyme to give 2-cyano-4-methyl-pentanoic acid ethyl ester of formula (iv);

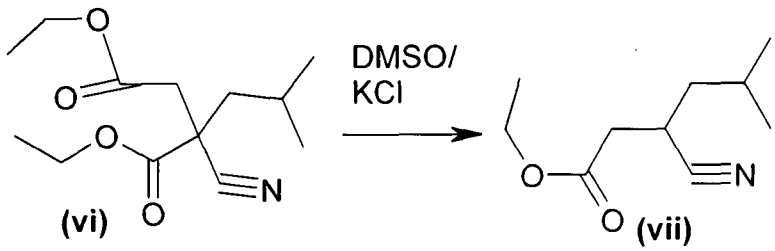



b) reaction of Compound of formula (iv) with halo acetic acid ethyl ester (v), wherein halo group include chloro, bromo and iodo, in presence of base such as alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride, in a polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4-dioxane, and dimethoxy ethane,

preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably dimethoxy ethane and dimethyl sulphoxide at temperature of about 10 to 80 $^0$C, preferably at 50 to 60 $^0$C to give diethyl 2-cyano-2-isobutylsuccinate (vi);

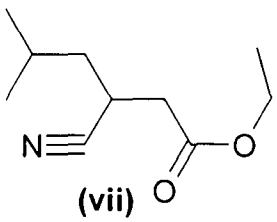



c) reaction of compound (vi) with potassium chloride or sodium chloride in an organic solvent such as dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get 3-Cyano-5-methyl-hexanoic acid ethyl ester (vii);

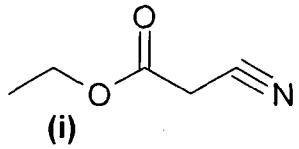



Such that at each step the intermediates were optionally isolated and purified with suitable process.

2. A process for synthesis of 3-cyano-5-methylhexanoic acid ethyl ester of formula (vii)



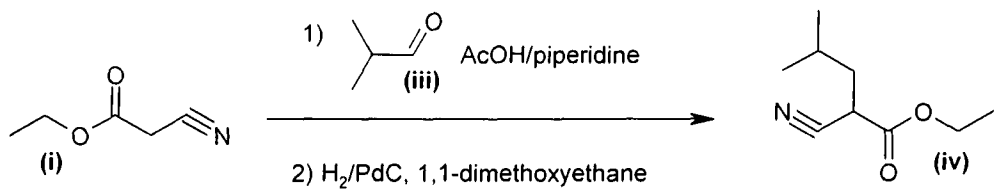

from ethyl cyano acetate of formula (i)



(i)

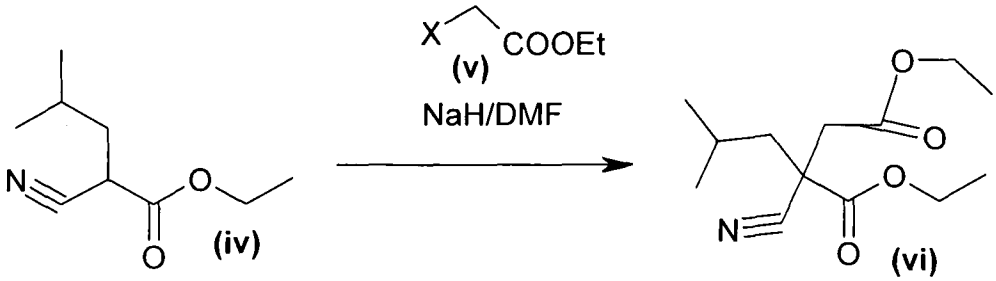
comprising,

a) condensation of 2-methyl-propionaldehyde with cyanoacetic acid ethyl ester in presence of base such as piperidinium acetate, and further hydrogenation using noble metal catalyst such as platinum oxide, palladium on carbon, Raney nickel and palladium hydroxide on carbon, preferably palladium on carbon and palladium hydroxide on carbon in polar solvent such as 1,4-dioxane, tetrahydrofuran, dimethoxy ethane and diglyme, preferably dimethoxy ethane and diglyme under hydrogen pressure of about 1 $kg/cm^2$ to 5 $kg/cm^2$, preferably about 2 $kg/cm^2$, further isolation of the product in solution form from the catalyst by filtration;

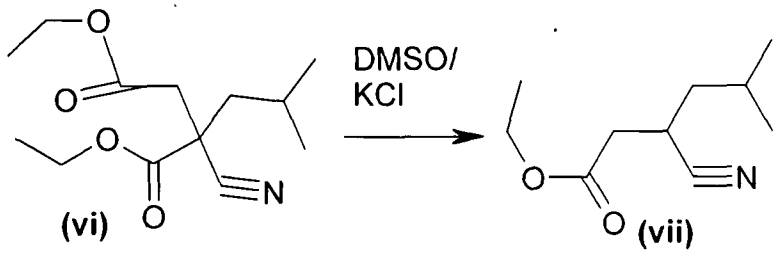



(i) (iii) (iv)

b) reaction of Compound of formula (iv) with halo acetic acid ethyl ester (v), wherein halo group include chloro, bromo and iodo, in presence of base such as alkali hydroxide sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride, in a polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4-dioxane, and dimethoxy ethane, preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably dimethoxy ethane and dimethyl sulphoxide at temperature of about 10 to 80 $^0C$, preferably at 50 to 60 $^0C$ to give diethyl 2-cyano-2-isobutylsuccinate (vi);

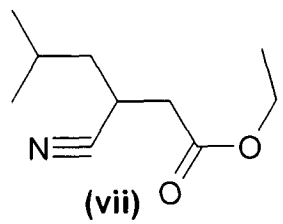



c) reaction of compound (vi) with potassium chloride or sodium chloride in an organic solvent such as dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get 3-Cyano-5-methyl-hexanoic acid ethyl ester (vii);

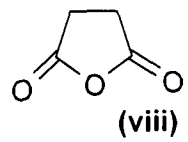



Such that at each step the intermediates were optionally isolated and purified with suitable process.

3. A process for synthesis of 3-cyano-5-methylhexanoic acid ethyl ester of formula (vii)

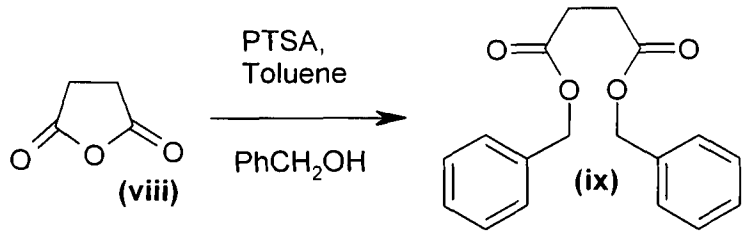



from dihydrofuran-2,5-dione of formula (viii)

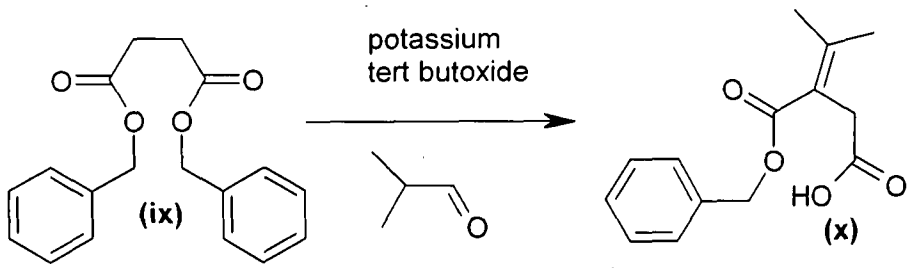



comprising,

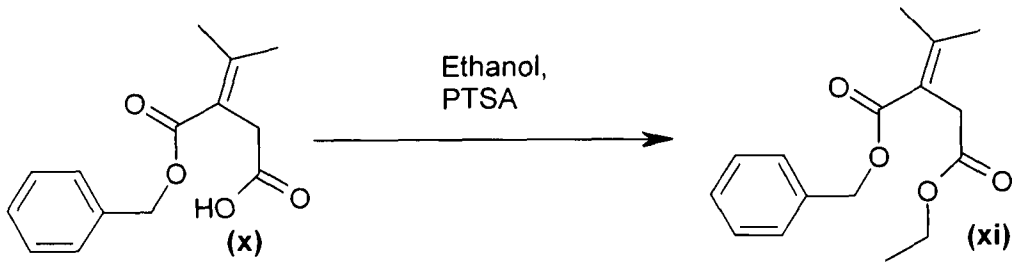
a. reaction of dihydrofuran-2,5-dione of formula (viii) with benzylalcohol in presence of paratoluenesIfonic acid to give succinic acid dibenzyl ester (ix);



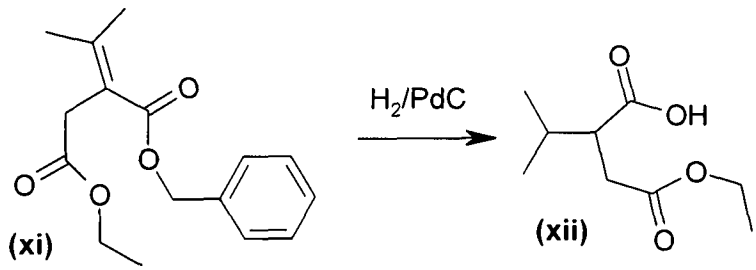

b. reaction of compound of formula (ix) with 2-Methyl-propionaldehyde in presence of a base selected from potassium *tert*-butoxide, sodium hydride, sodium ethoxide, sodium methoxide, preferably potassium *tert*-butoxide in a polar solvent selected from *tert*-butanol, tetrahydrofuran, dimethyl sulfoxide and dimethoxy ethane, preferably *tert*-butanol at temperature of about 25 to 80 $^0$C, preferably at 50 to 60 $^0$C to give 2-Isopropylidene-succinic acid 1-benzyl ester of formula (x);



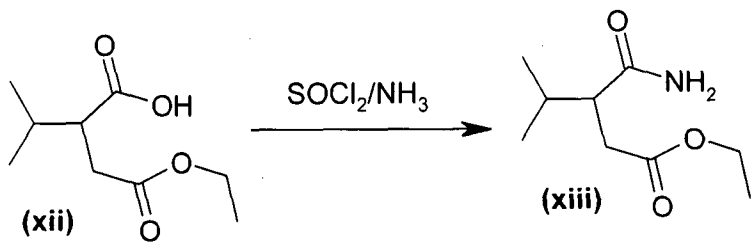

c. acid catalyzed esterification of compound of formula (x) with aliphatic alcohol selected from straight chain or branched, of $C_1$ to $C_6$ carbon chain length, preferably ethanol, wherein acid used is aryl sulfonic acid such as *para*-toluenesulfonic acid to get 2-isopropylidene-succinic acid 1-benzyl ester 4-ethyl ester (xi);



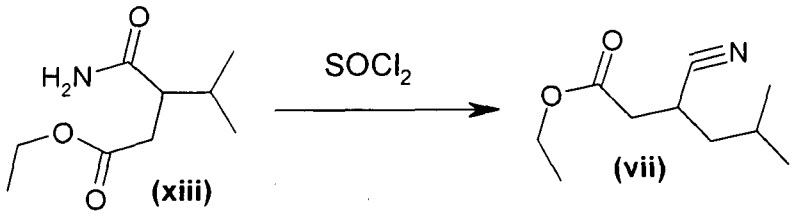

d. hydrogenation and hydrogenolysis of compound of formula (xi) in presence of noble metal catalyst selected from platinum oxide, palladium on carbon and palladium hydroxide on carbon; preferably the noble metal catalyst is palladium on carbon and palladium hydroxide on carbon in polar solvent such as methanol, ethanol, *n*-butanol, preferably ethanol at hydrogen pressure maintained between 3 kg/cm$^2$ to 15 kg/cm$^2$, preferably about 10 kg/cm$^2$ to get 2-isopropyl-succinic acid 4-ethyl ester (xii);




e. reaction of compound of formula (xii) with thionyl chloride to give acid chloride *in situ* treatment of acid chloride with ammonia to get 3-carbamoyl-5-methyl-hexanoic acid ethyl ester (xiii);

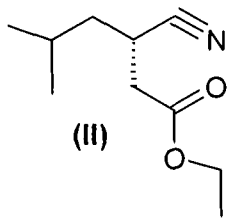



f. dehydration of compound of formula (xiii) in presence of dehydrating agent such as thionylchloride, phosphorous pentoxide, phosphorous oxychloride to get 3-Cyano-5-methyl-hexanoic acid ethyl ester (vii);

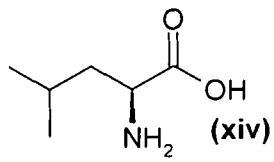



Such that at each step the intermediates were optionally isolated and purified with suitable process.

4. A stereoselective process for synthesis of (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II)

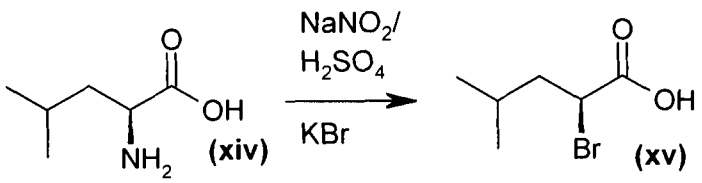



from *S*-leucine of formula (xiv)

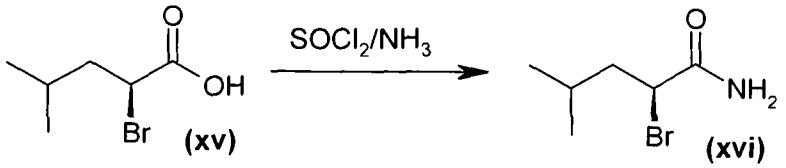



comprising,

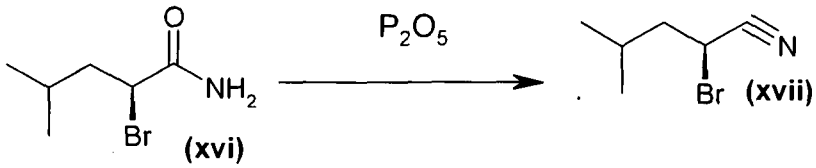
a) Samdmeyer reaction of (*S*)-2-amino-4-methyl-pentanoic acid of formula (xiv) using sodium nitrite and sulfuric acid followed by treatment of reaction mass with potassium bromide to get (*S*)-2-bromo-4-methyl-pentanoic acid (xv);



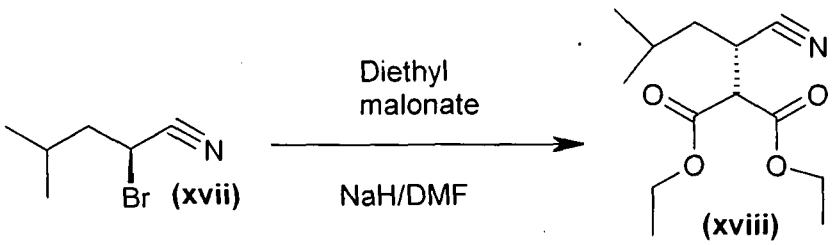
b) reaction of compound of formula (xv) with thionyl chloride to get acid chloride, treatment of which ammonia *in situ* to get (*S*)-2-bromo-4-methyl-pentanoic acid amide (xvi);



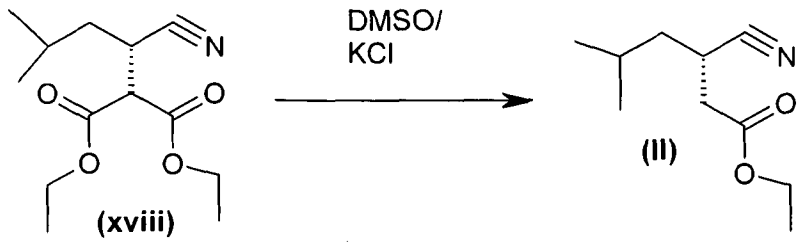

c) dehydration of compound of formula (xvi) in presence of dehydrating agene such as phosphorous pentoxide to get (*S*)-2-bromo-4-methyl-pentanenitrile (xvii);




d) preparation of enantiomerically enriched 2-((*S*)-1-cyano-3-methyl-butyl)-malonic acid diethyl ester (xviii) by $S_N2$ displacement reaction of compound of formula (xvii) with diethyl malonate in presence of base such as sodium hydride, sodium ethoxide, sodium methoxide and potassium carbonate, preferably sodium hydride and polar solvent selected from N, N-dimethyl formamide, tetrahydrofuran, 1,4 dioxane, and dimethoxy ethane; preferably dimethoxy ethane and N, N-dimethyl formamide, more preferably dimethoxy ethane at temperature of about 10°C to 80°C, preferably at 50°C to 60°C;

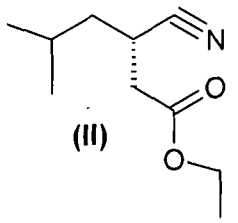



e) decarboxylation of compound of formula (xviii) in presence of potassium chloride or sodium chloride in presence of solvent such dimethylsulfoxide at temperature of about 140°C to 180°C, preferably at 150°C to 160°C to get enantiomerically pure (*S*)-3-cyano-5-methyl-hexanoic acid ethyl ester (II).




Such that at each step the intermediates were optionally isolated and purified with suitable process.

5. A process for enantiomeric enrichment of (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II)




in its racemate or partially enriched form comprising stereoselective hydrolysis of *R* isomer of 3-cyano-5-methylhexanoic acid ethyl ester involving enzymatic kinetic resolution technique using lipase enzyme derived from the microorganism *Candida antarctica B* in presence of buffer at concentration from about 10 mM to 100mM, preferably 30 mM to 60 mM and more preferably 40 mM at temperature range from 10°C to 50°C, preferably at 15°C at pH ranging from 6 to 8, preferably at pH 7.2 and isolation of un-hydrolyzed (*S*) 3-cyano-5-methylhexanoic acid ethyl ester of formula (II).

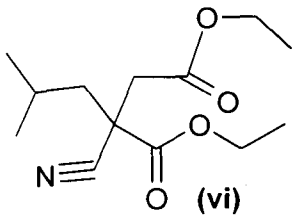
6. The process as claimed in claim 5 wherein pH is adjusted using the base such as potassium hydroxide, sodium hydroxide and ammonium hydroxide.

7. The process as claimed in claim 5 wherein, the hydrolyzed *R* isomer of 3-cyano-5-methylhexanoic acid is recycled to obtain racemic 3-cyano-5-methylhexanoic acid

ethyl ester which was then further subjected to kinetic enzymatic resolution to obtain *S*-3-cyano-5-methylhexanoic acid.

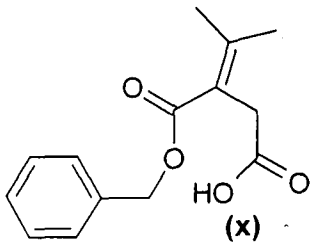
8. The process as claimed in claim 7 wherein, the recycling of *R* isomer of 3-cyano-5-methylhexanoic acid is carried out by converting it to its ethyl ester and further subjecting the ethyl ester to racemization in presence of base in an alcoholic solvent.

9. A compound 2-Cyano-2-isobutyl-succinic acid diethyl ester of formula (vi)

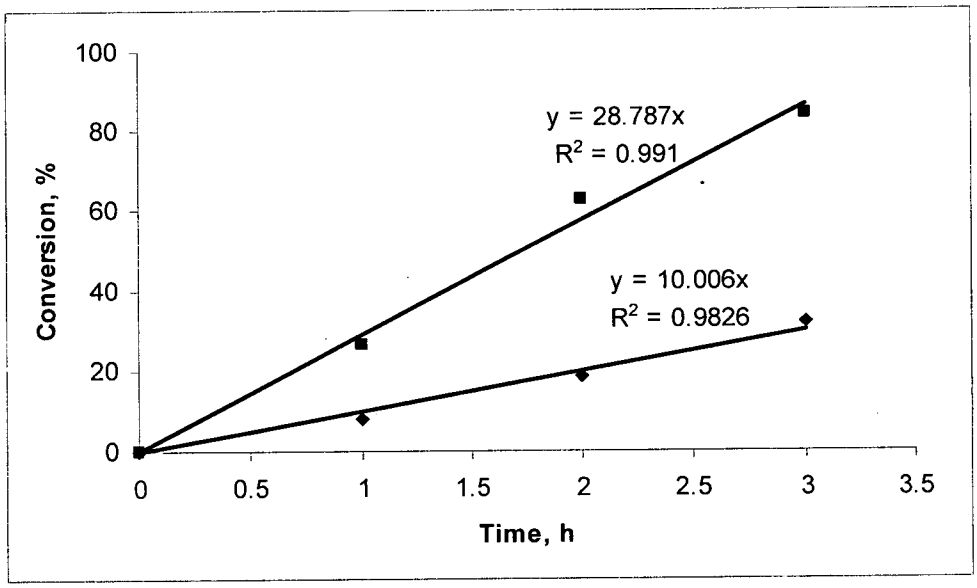


**(vi)**

10. A compound 2-Isopropylidene-succinic acid 1-benzyl ester of formula (x)

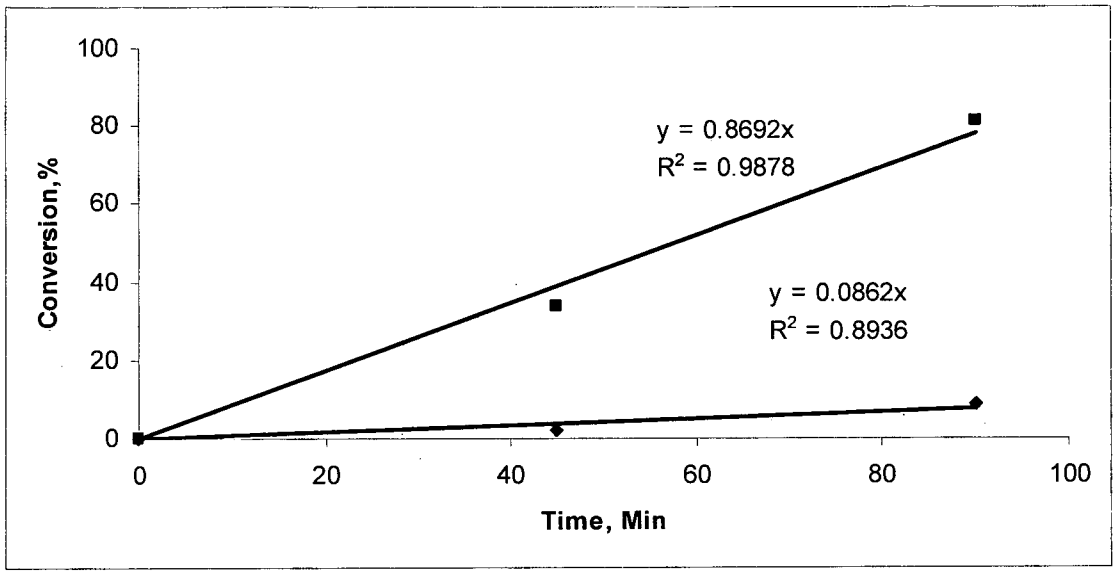


**(x)**




♦*(S)* - 3-cyano-5-methylhexanoic acid, ■*(R)* - 3-cyano-5-methylhexanoic acid

**Figure 1**




♦*(S)* - 3-cyano-5-methylhexanoic acid, ■*(R)* - 3-cyano-5-methylhexanoic acid

**Figure 2**