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(54) Title: A NOVEL PROCESS FOR SYNTHESIS OF PREGABALIN FROM SUBSTITUTED CYCLOPROPANE INTERMEDIATE AND A PROCESS FOR ENZYMATIC RESOLUTION OF RACEMIC PREGABALIN

(57) Abstract: A novel process of preparation of racemic pregabalin from comprising an intermediate of structure 111 comprising reductive ring opening to form a structure IV followed few alternative routes that yield racemic pregabalin that is resolved to yield (S) + pregabalin by using chemical or enzymatic methods. A new chemical entity of structure IHc lilc is also disclosed as a novel intermediate. A part of the novel process also comprises a new process of producing a compound of structure VII comprising acylation of compound of structure V followed by elimination and improving a process of producing a compound of structure VII comprising reacting isovaleraldehyde with nitromethane through the compounds 4-methyl-1-nitropentan- 2-ol, 3-methyl-1-(nitromethyl)butylacetate, and 3-methyl-1-(nitromethyl)butyl acetate under suitable conditions to 4-methyl-1-nitropet-1-ene. Enzymatic processes of resolution of racemic pregabalin esters into (S) pregabalin and (R) pregabalin are also disclosed.

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A NOVEL PROCESS FOR SYNTHESIS OF PREGABALIN FROM SUBSTITUTED CYCLOPROPANE INTERMEDIATE AND A PROCESS FOR ENZYMATIC RESOLUTION OF RACEMIC PREGABALIN.

TECHNICAL FIELD

The present invention is directed to new "substituted cyclopropane" intermediate for synthesis of racemic pregabalin. The present invention also relates to a method for stereoselective bioconversion of racemic 3-aminomethyl-5-methylhexanoic acid alkyl ester to 3-aminomethyl-5-methylhexanoic acid.

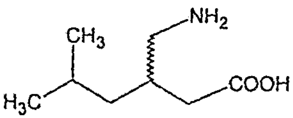
BACKGROUND OF THE INVENTION

Pregabalin is a Gamma-aminobutyric acid (GABA) analogue that is reported in various patents to prevent and treat a range of diseases.

Application of pregabalin has been disclosed in US 5563175 in treating seizure disorders, in US 6127418 to dramatically reduce the gastrointestinal damage caused by drugs and alcohol, treat the conditions resulting from ethanol withdrawal syndrome, and GI disorders characterized as inflammatory bowel disorders, functional bowel disorders, dyspepsia, visceral pain; in US 6194459 for treating gastrointestinal damage and disorders and a composition comprising a GABA analog and a non-steroidal anti-inflammatory drug; in US 6194459 for treating physiological conditions associated with the use, or sequelae of use, of psychomotor stimulants including cocaine, amphetamine and like addictive drugs/substances; in US 6242488 for preventing or treating pain comprising a GABA analog and a non-steroidal anti-inflammatory drug such as a composition comprising pregabalin and naproxen sodium; in US 6306910 for treating insomnia; in US 6326374 for eliciting an enhanced analgesic response comprising administering an analgesically effective amount of a GABA analog; and caffeine; in US 6329429 for preventing and treating inflammatory diseases; in US 6359005 for treating mania and treating and preventing bipolar disorder; in US 6372792 for preventing or treating alcoholism, irritable bowel disorder or irritable bowel syndrome; in US 6451857 as an anti-epileptic compound; in US 6566400 for treating physiological conditions associated with the use, or sequelae of use, of psychomotor stimulants including cocaine; in US 6593368 for treating acute pain by a combination, comprising a synergistic amounts of gabapentin and celecoxib; in US 6620829 for treating non-inflammatory cartilage damage; in US 6680343 for treating renal colic; in US 6887902 for treating inflammatory diseases arthritis and rheumatoid arthritis; in US 6992109, for treating urinary incontinence; US 7026505 for treating tinnitus. Pregabalin is also shown to be effective in combination with NMDA receptor antagonists (US 6942876), in buccal sprays (US 6977070); as lactose conjugates (US 7022678) in combination with 2-(7-chloro-1,8-naphthyridin-2-yl)-3-(5-methyl-2-oxo-hexyl)-1-isoindolino-ne or 2-(7-chloro-1,8-naphthyridine-2-yl)-3-(5-methyl-5-hydroxy-2-oxohexyl-)-1-isoindolinone for obsessive-compulsive disorder (US 7026332); and can be gave as an ingredient of a liquid pharmaceutical composition. Many more examples not mentioned here exist and may be added in future on applications of pregabalin.

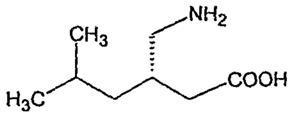
The therapeutic effects of pregabalin including seizure suppressive effect while avoiding the undesirable side effect of ataxia is attributed to the S-enantiomer, the racemic compound (.+-.)-3-(aminomethyl)-5-methylhexanoic acid (also known as racemic isobutyl-GABA or also known as Pregabalin). With the S-enantiomer having better anticonvulsant activity than the R-enantiomer, the commercial utility of pregabalin requires an efficient method for directly preparing the S-enantiomer substantially free of the R-enantiomer or comprises following up of the synthesis process that results in a racemic mixture with a method of resolution of the two enantiomers from each other.

Pregabalin, 3-(aminomethyl)-5-methylhexanoic acid, is a compound, a racemic mixture of which is indicated as having chemical Structure (I)



I

It is also titled as CI-1008 assigned by Park and Davis. It has γ-aminobutyric acid functionality, which is considered to be responsible for the desired activity. S-pregabalin having structure Ia, given in the following,



Ia

is useful as an anticonvulsant because it has been found to activate GAD (L-glutamic acid decarboxylase) promoting the production of gamma-aminobutyric acid (GABA), one of the brain's major inhibitory neurotransmitters. It is expected to show several times higher activity than Neurontin. Pregabalin has analgesic as well as anxiolytic activity.

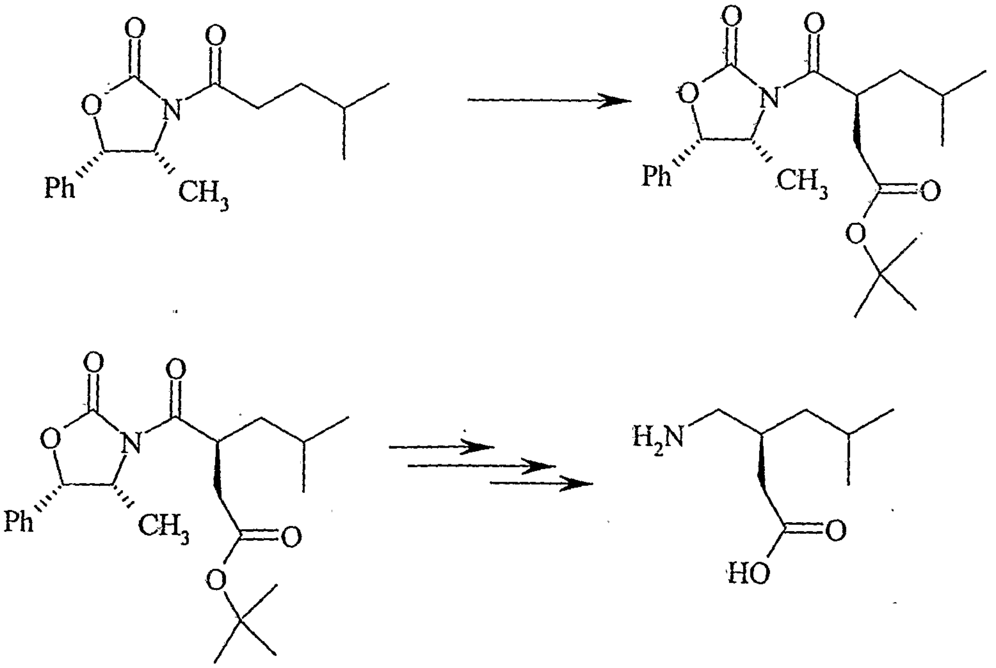
Several processes for synthesis of (S)-(+) pregabalin are known, some of which are given in DRUGS OF FUTURE, 24 (8), 862-870(1999). One such process includes 1,3-isobutyl glutaric acid conversion into corresponding anhydride, by treatment with refluxing acetic anhydride. The monoamide formed after NH_4OH treatment is resolved and subjected for Hoffmann degradation provides (S)-(+) pregabalin.

This invention embodies a novel process that comprises cyclopropane ring formation as intermediate and followed by ring opening by reduction to yield racemic pregabalin while the bioconversion yields the desired active isomer.

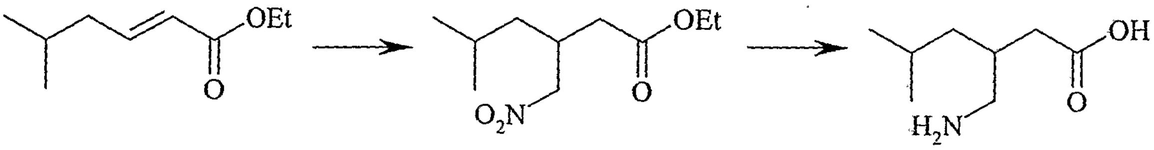
Several methods covered in literature mention optical resolution of racemic mixtures by partial recrystallization. The most common agents for such resolution of racemic mixtures are (S)-(+) Mandelic acid and l- phenylethylamine. Apart from this there are many patents comprising of use of chiral reagent for preparation of stereoselective (S)-isomer.

This invention embodies novel processes for preparation of enantiometrically pure pregabalin from various precursors such as its esters by enzymatic hydrolysis, bioconversion for obtaining (S)-(+) pregabalin by use of enzymes.

US patent No. 5563175, U.S. 5,599,973, US 5,563,175, U.S.5,684,189, U.S. 6,197,819, U.S.2003225161, WO9209560 and WO9323383 disclosed synthesis of pregabalin using azide as an intermediate. The enantioselectivity of the pregabalin is achieved by 4-methyl 5-phenyl oxazolidinone ring formation. The process disclosed comprises the steps of forming an acid chloride of an acid of the formula HOC(= O)CH(R_1)(R_2)-(where wherein R_1 is a straight or branched alkyl of from 1 to 6 carbon atoms, phenyl, or cycloalkyl having from 3 to 6 carbon atoms; R_2 is hydrogen, methyl, or carboxyl) the corresponding acid chloride having the formula ClC(= O)CH(R_1)(R_2), adding the acid chloride to a solution of (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone and n-butyllithium at -78.degree. C. under argon to produce an oxazolidinone derivative, treating the oxazolidinone derivative with benzyl .alpha.-bromoacetate to produce an ester, treating the ester with hydrogen peroxide and lithium oxide followed by treatment with sodium metabisulfite to produce a compound, treating the compound with borane dimethyl sulfide complex to produce the corresponding tosylate from the compound, forming an azide from the corresponding tosoylate and reducing the azide to the amine. In general, this method can be represented by following route for steriospecific pregabalin synthesis;



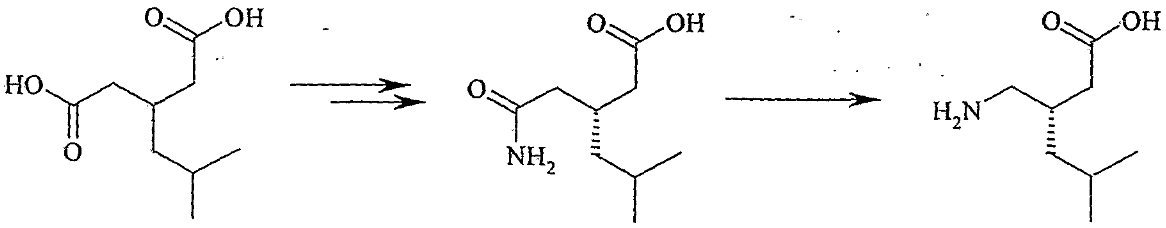
this method involves nine steps in the synthesis and enantioselectivity is obtained by using chiral reagent like oxazolidinone along with some hazardous reagents like N-BuLi (n-butyllithium), LiOH. However, this process is limited to small scale and is not practical for large-scale synthesis because it employs costly reagents which are difficult to handle. In another prior art method, 3-alkyl-4-aminobutanoic acid is prepared via Michael addition between nitromethane and 2-alkenoic ester followed reduction of nitro group to amine with 10% palladium on carbon (Andruszkiewicz et al., Synthesis, 1989, 953) by following reaction.



This prior art differs from the process of this invention that comprises preparation of nitroalkene and Michael addition type reaction of sulfur ylide.

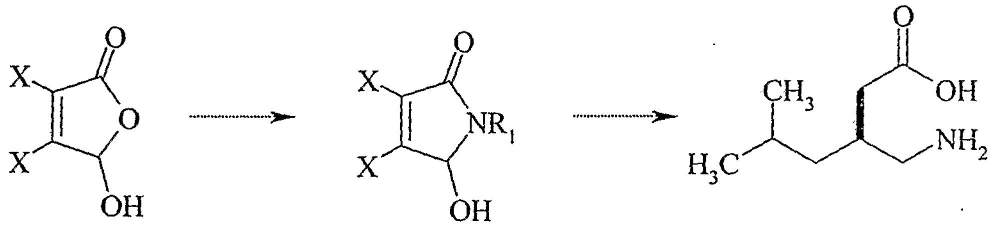
According to the US patent no. 5637767 and 5840956, 3-(aminomethyl)-5-methylhexanoic acid is prepared in four steps from isovaleraldehyde that include condensation, cyanide addition, selective monocarboxylation and reduction of cyanide to amine followed by resolution with (S)-mandelic acid. This prior art method comprises use the hazardous metal cyanides as amine source. The process of this invention avoids use of the toxic metal cyanide as it proceeds through cyclopropane ring opening metathesis using nitromethane as amine source.

Yet another process disclosed in patent U.S. 5629447 and U.S. 5616793 follows the same path that includes opening of cyclic anhydride with ammonia followed by resolution and then Hoffmann rearrangement lead to (S)-(+)-3-aminomethyl-5-methylhexanoic acid as per following route of reaction:

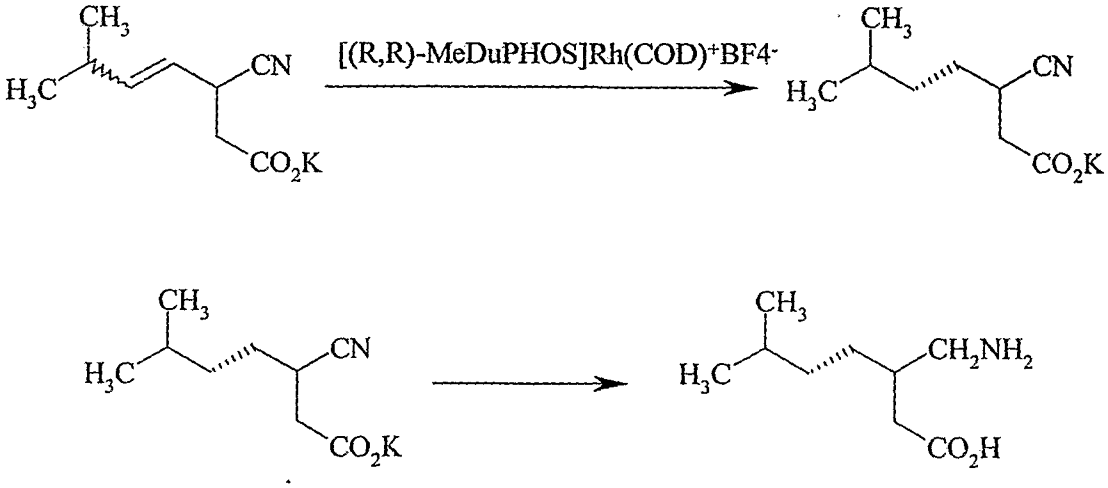


The process of this invention, as distinct from the prior art process, does not involve Hoffmann rearrangement and resolution but the bioconversion of 2-aminomethyl-5-methylhexanecyanide will directly gives the pure S-enantiomer.

According to the patent WO 03/093220, pregabalin was prepared from highly substituted butyrolactams. Butyrolactams were prepared from reductive amination of mucobromic acid by ammonium formate. Route of reactions used is shown in the following :



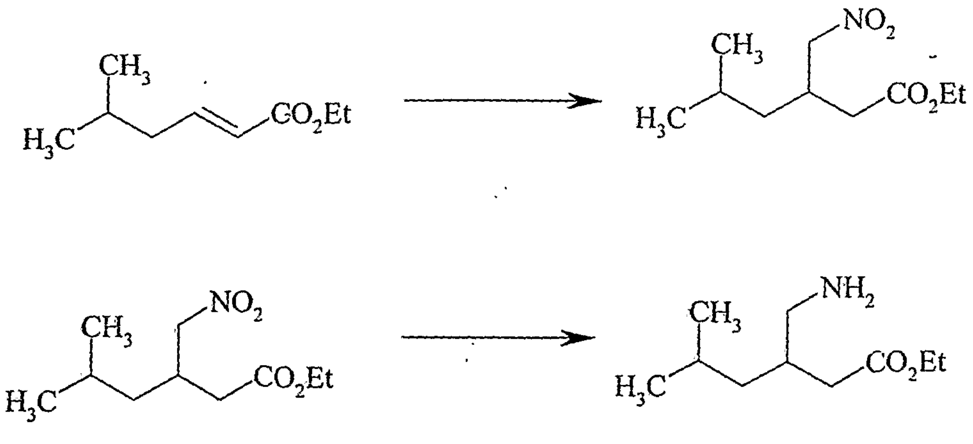
This prior art process is very much different than the process of this invention that comprises reductive cyclopropane ring opening. In yet another process for enantioselective synthesis of pregabalin is an asymmetric hydrogenation process. The process mentioned in U.S. 6891059 uses chiral catalyst [(R,R)-MeDuPHOS]Rh(COD)BF_4]for asymmetric hydrogenation of ethyl-3-cyano-5-methylhex-3-enoate to afford enantiomerically pure isomer of pregabalin. This patent also mentions the use of sponge Ni for stereoselective reduction of cyanohexenoate ester. Route of reaction used is as follows:



Process of this invention, as distinct from this prior art, comprises reduction of nitro group by simple Raney Ni, and stereochemistry is imparted by bioconverion of nitrile group to acid.

Burk et al., in WO 99/31041 and WO 99/52852, describes the asymmetric hydrogenation of .beta.-substituted and .beta.,.beta.-disubstituted itaconic acid derivatives to provide enantiomerically enriched 2-substituted succinic acid derivatives which is intermediate precursor for pregabalin.

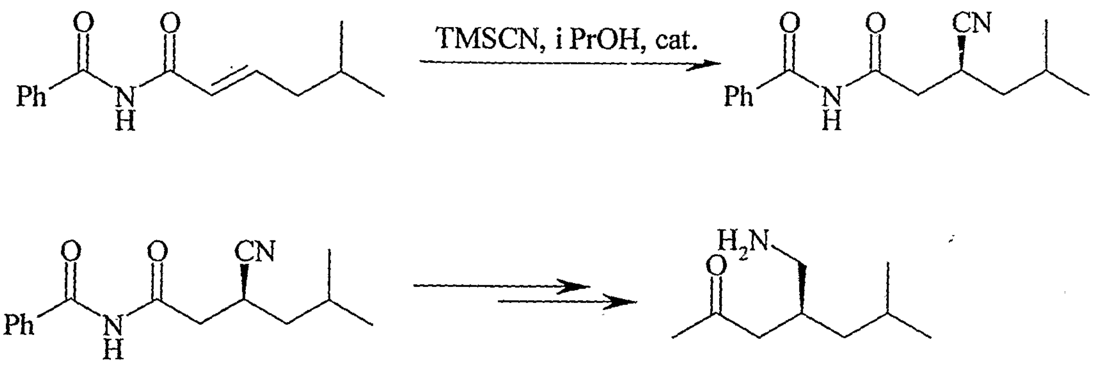
The patent U.S. 7141695 discloses the synthesis and use of substituted acrylic acid esters for synthesis of pregabalin as well as gabapentin. The process proceeds through lactam intermediate followed by ring opening by hydrolysis. This process provides racemic pregabalin and the process is shown in the following:



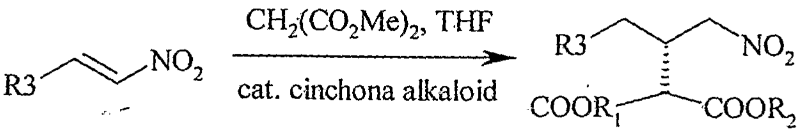
Patent US 6488964 refers to the manufacture of coated pregabalin.

The method mentioned in patent WO 2003/104184 discloses method for synthesis of acyloxy derivatives of GABA analogues.

A process published by G. M. Sammis, et al., J. Am. Chem. Soc. 125(15), 4442-3(2003) takes advantages of the asymmetric catalysis of cyanide conjugate addition reactions. The method discloses the application of aluminium salen catalyst to the conjugate addition of hydrogen cyanide to α,β-unsaturated imides . Reportedly TMSCN is useful source of cyanide that can be used in place of hydrogen cyanide. The route of reaction is given below:



Recent studies (Li, et al., J. Am. Chem. Soc., 126(32), 9906-07 (2004). have indicated that cinchona alkaloids are broadly effective in chiral organic chemistry. A range of nitro alkenes were reportedly treated with dimethyl or diethyl malonate in tetrahydrofuran in presence of cinchona alkaloids to provide high enantiomeric selectivity as shown below:

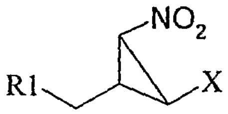


Where R3 may be several alkyl; aryl groups

The above mentioned prior art processes use (a) chiral auxillary such as oxazolidinone and proceed through azide intermediate with several steps, or (b) make use of asymmtric reduction of cyano hexenoate derivative, or (c) proceed thorough anhydride formation from glutaric acid followed by ring opening with ammonia and Hoffmann rearrangement and resolution.

SUMMARY OF THE INVENTION

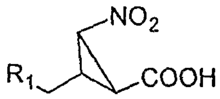
The method of this invention proceeds through an entirely different route that comprises formation of a di-substituted cyclopropane ring, shown as structure III in the following, which in this invention is used for the first time as a precursor of pregabalin:



III

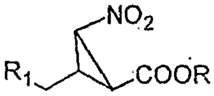
wherein, R1=Alkyl, Aryl, allyl, heterocycle, straight chain or branch chain, X= -CN or -COOR, wherein R may be hydrogen or straight chain or branched chain alkyl or aryl group.

When in a compound of structure III R is H, and R_1 = -alkyl, aryl, heterocycle, straight chain or branch chain, the compound is of structure IIIa,



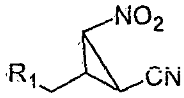
IIIa ,

When in a compound of structure III, and R is straight chain or branched chain alkyl or aryl group and and R_1 = -alkyl, aryl, heterocycle, straight chain or branch chain, the compound is of structure IIIb,



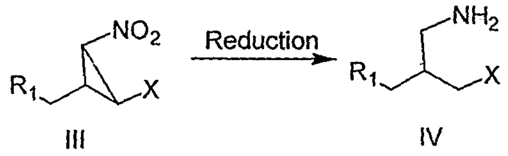
IIIb

When in a compound of structure III, X = -CN, the compound is a new chemical entity and and R_1 = -alkyl, aryl, heterocycle, straight chain or branch chain, the compound is of structure IIIc



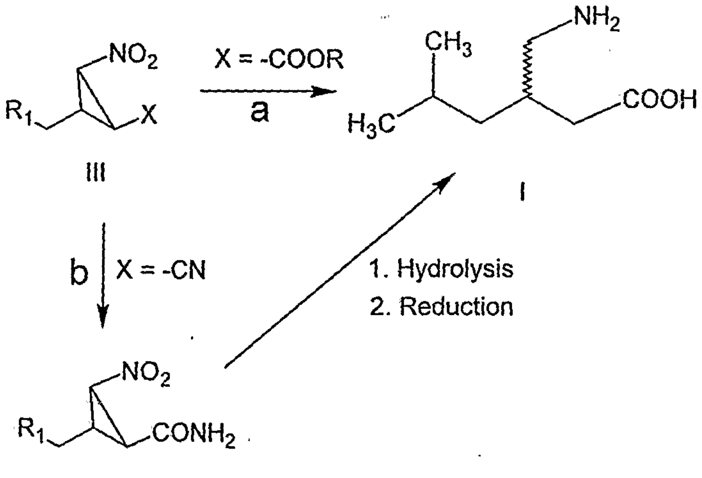
IIIc

First step of one embodiment of this invention to prepare pregabalin comprises reductive ring opening of compound III to form a compound of structure IV ; the said reductive ring opening being achieved using reducing agent like Raney Ni/ H_2 or Pd/C or Zn/$HCOONH_4$ or Zn/HCOOH or Fe/CH_3COOH or Fe/HCl at 25 °C in polar solvent:

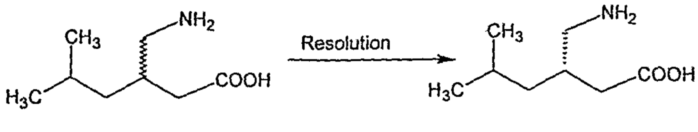


When R1 = isopropyl group and X = -COOH, the result of the reaction is formation of a racemic mixture of pregabalin. When X = -COOR wherein R is straight or branch chain alkyl group then there is one more step of hydrolysis of ester to corresponding acid of structure IIIa.

When R1 = isopropyl group and X= -CN , the reaction also results into racemic pregabalin by route (b) comprising a step of conversion of -CN to –C(O)NH2 (nitrile to amide) followed by reduction and hydrolysis of the amide group to carboxylic acid group. The alternative routes available for such a production of racemic mixture from a compound of structure III are summarized below:

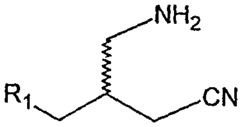


The racemic mixture formed by chemical process can be subjected to a prior art process of resolving them to get (S)-(+) pregabalin, including use of agent for resolution like (S)-(+)-mandelic acid in IPA /Water or (+)-α-phenyl ethylamine in ethanol/chloroform or *l* –ephedrine:



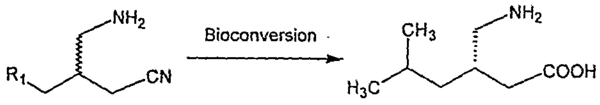
I

Ia



IVa

When X= -CN, and R1 = isopropyl group in a compound of structure IVa, , this invention opens up and shall include in it, another possibility of subjecting the compound of structure IVa having nitrile group at first carbon atom to the action of nitrilase that may regioselectively as well as sterioselectively convert nitrile group to carboxyl group by enzymatic action of nitrilase leading to production of (S)-(+) pregabalin as the only isomer as shown below:

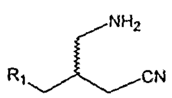


IVa

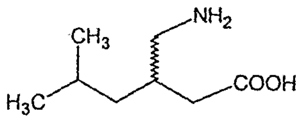
Ia

Structure of IVa is prepared by reduction of i compound of structure IIIa to compound IVa by Fe/acetic acid or Fe/HCl, Raney Ni,/H_2 as reducing agent.

Where X = -COOR wherein R is straight or branched chain alkyl group and R1 = isopropyl group compound of structure IVa, another possibility of subjecting the compound of structure IVa having carboxyl group, wherein X is as defined at first carbon atom, to the action of a suitable enzyme including but not limited to, hydrolases, further including esterases, lipases or proteases that may regioselectively as well as sterioselectively convert ester group to carboxyl acid group by enzymatic action of lipases or proteases leading to production of (S)-(+) pregabalin. Protocol for regioselctive conversion by an esterase or a hydrolase of a (R) pregabalin ester has been demonstrated in the following. The method of use of enzyme is so generic to all enzymes that. it is obvious that an enzyme capable of converting _compound of structure IVa here X = -CN will work with same protocol or by modifications that are within the scope of a person skilled in the art.Thus, a process of conversion of a compound



of structure IV to racemic pregabalin of structure I



I , by enzymatic conversion of _-CN group at first carbon atom by the action of nitrilase that is capable of regioselectively as well as sterioselectively convertion of nitrile group to carboxyl group. By enzymatic action of an enzyme capable of stereoselctive conversion of the compound of structure IVa to (S)-(+) pregabalin as the only isomer may comprise following steps:

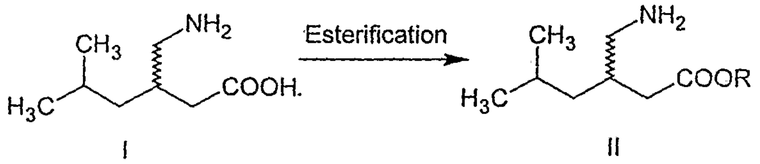
a. A compound of structure IVa as given above is dissolved in aqueous sodium phosphate buffer (about 50 mM, about pH = 7) containing varying amount of at least one water miscible organic solvent,

b. the enzyme capable of stereoselective conversion to (S) pregabalin is added and shaken for a time period and at a temperatures range sufficient for conversion of substantial portion of R- 3-aminomethyl-5-methylhexanoic acid methyl ester to 3-aminomethyl-5-methylhexanoic acid.

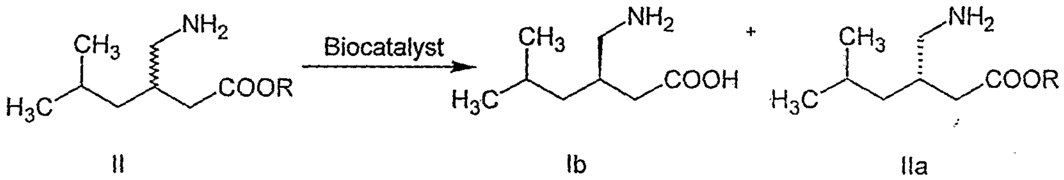
In this process, water miscible organic solvent shall vary between 0.1 % - 40 %, v/v, and included at least an acetonitrile, N,N-dimethyl formamide, dimethyl sulphoxide, or tetrahydrofuran,; time period of shaking is about 6 -18 h, and the temperature is kept within the range of about 20 °C - 60 °C. The enzyme used may be a nitrilase.

In one embodiment the present invention provides 99.5 % enantiomeric excess of (S)-3-aminomethyl-5-methylhexanoic acid by using an appropriate enzyme. In another embodiment, the present invention provides (R)-3-aminomethyl-5-methylhexanoic acid with more than 98% enantiomeric excess by using an appropriate enzyme for (R) enantiomeric selectivity.

A further embodiment of the present invention provides process for preparation of pregabalin alkyl esters of structure II wherein R is preferably methyl or more preferably ethyl, or any linear chain or branch chain alkyl group .

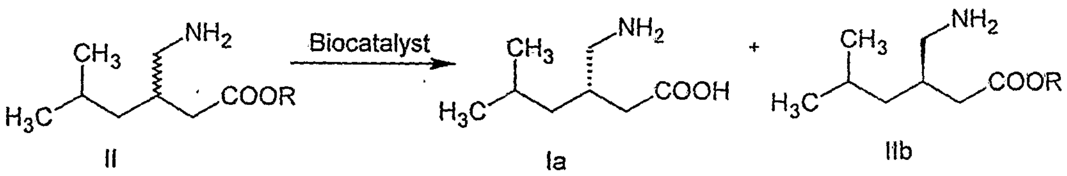


A further embodiment of the present invention provides process for stereoselective hydrolysis of alkyl esters of pregabalin to R-isomer of pregabalin by enzymes esterases, lipases and proteases either in free form or in immobilized form (scheme A)



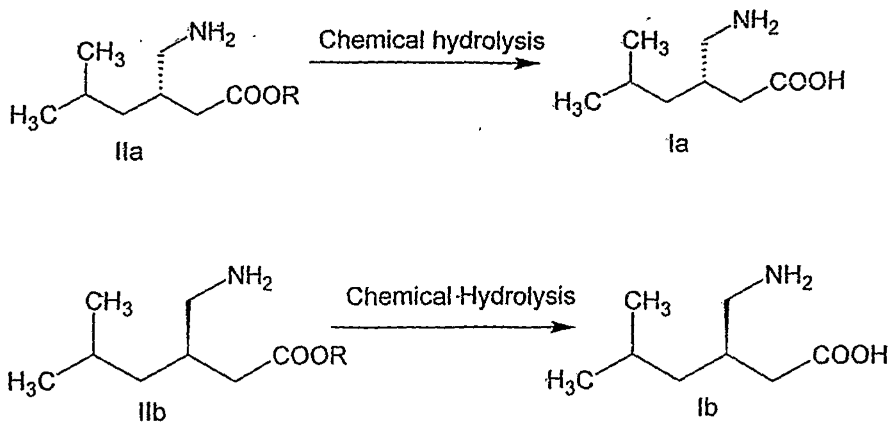
Scheme A

In yet another embodiment the present invention provides stereoselective hydrolysis of alkyl esters of pregabalin to S-isomer of pregabalin of structure (Ia) by enzymes esterases, lipases and proteases capable of selectively acting on (S) isomer of structure II, the said enzyme being either in free form or in immobilized form (Scheme B).



Scheme B

In yet another embodiment the present invention provides process for chemical hydrolysis of S-Pregabalin alkyl esters or R-Pregabalin alkyl ester to corresponding S-Pregabalin or R- Pregabalin as shown in scheme C

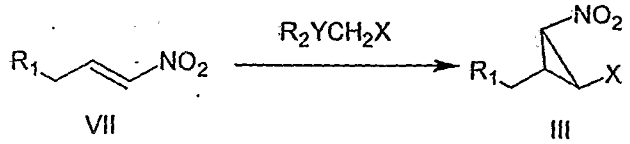


Scheme C

In embodiments where enzyme is used, the reaction medium may contain one or more of a water immiscible organic solvent. In a preferred embodiment here, acetonitrile has been used, although it may be replaced by any other water immiscible solvent/s capable of discharging same function. Such a water immiscible solvent may include, witout limitation, acetonitrile, N,N-dimethyl formamide, dimethyl sulphoxide or tetrahydrofuran. I agree with You but We don't have any data of solvent study except Acetonitrile

.A further embodiment of this invention comprises use of enzyme catalyst isolated from microorganisms. Yet further embodiment of this invention comprises isolation of the said enzyme from a microorganism of one or more of a group including, but not limited to, Rhodococcus species, Pseudomonas species, Arthrobactor species, Bacillus species and Aspargillus nigar.

There are several methods available in prior art for achieving synthesis of asymmetric cyclopropane ring (Marian Mikolajczyk, Pure and applied chemistry 2005, 77; Armando Córdova *et a* l, (Advanced Synthesis and Catalysis, 2007, 349, 1028-1032; Jurgen Zindel *et al*, (Tetrahedron Letters, 1993, 34, 1917-1920); G. H. Kulkarni, SYNTHESIS 1995, 1545-1548, G. B. Payne, J. Am. Chem. Soc. 1967, 32, 3351-3355). The method of preference in this invention for achieving synthesis of asymmetric cyclopropane ring formation comprises its formation from nitroalkene of structure VII and sulphurylide through Michael type addition reaction as shown in the following:

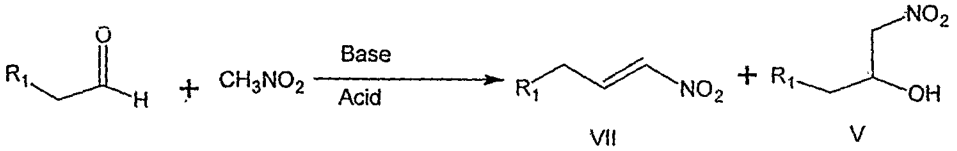


Wherein, R_2 = Alkyl, aryl, Y = P or S, and X= -CN or -COOR, wherein R may be hydrogen or straight chain or branched chain alkyl or aryl group..

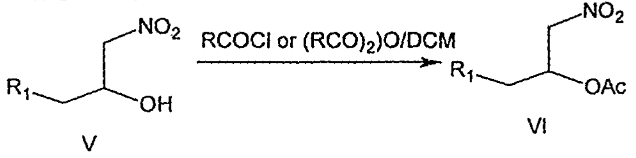
The 4-methyl-1-nitropent-1-ene of Structure (VII) when treated with sulphur or phoshphorus ylide under reflux temperature in chlorinated solvent preferably in dichloromethane affords the compound of structure III.

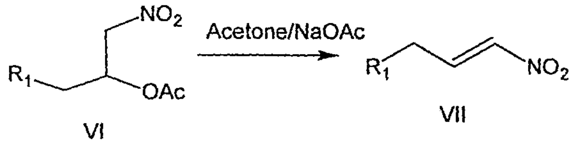
Sulphur or phosphorus ylide can be prepared preferably by a prior art process comprising mixing ethyl bromoacetate and dimethylsulfide with or without solvents, more preferably without solvent. Thereafter neutralizing the sulfonium salt by using saturated potassium carbonate and NaOH solution at 0-25°C.

Preferably the compound of Structure VII is prepared by known standard procedure as mentioned by Kulkarni (1995) (Synthesis 1995, p. 1545). Under basic condition preferably 50% aqueous NaOH or more preferably sodium metal in ethanol, or more preferably methanolic sodium hydroxide, isovaleraldehyde and nitromethane were stirred for about three hour to get a mixture of compound of structure IV and compound of structure V as shown in the following reaction:



In a further embodiment of this invention the compound of structure V is converted to compound of structure VII by acylation followed by elimination. Acyl chloride or anhydride especially acetyl chloride is the acylating reagent in chlorinated solvents like chloroform, dichloromethane, ethylene dichloride. Base selected for elimination consist of anhydrous sodium acetate or sodium carbonate in dry solvents like THF or acetone





Thus, a new process for synthesis of racemic as well as (S+) pregabalin starting from a compound of structure VII passing through compound of structure III and the same is also an embodiment of this invention .

DETAILS OF THE INVENTION

The following examples are not to be construed as limiting the scope of invention but are to be construed as illustrative only in nature. Additional variations within the concept of this invention that shall be evident from the contents of this specification to a person skilled in the art are also included within the scope and content of this specification. Although not mentioned separately in description to avoid repetition, all the details given in the examples are considered incorporated in the description.

Example 1 given below gives a method that directly affords 50 % nitroalkene if we use aqueous sodium hydroxide as a base in 1st step. The product is purified by column chromatography using silica gel column and cyclohexane and ethylacetate (9:1) as elution phase.

Examples 1a, 1b and 1 c together constitutes a modified method for preparation of nitroalkene, which is in three steps but provides better yield. We are getting 87% yield of nitroalcohol from method (1a + 1b +1c), while in method of Example 1 described below, it is only ~50% and also needs purification step. This 87% nitroalcohol is then converted into ester (Example 1b), and followed by elimination step to nitroalkene (example 1c). The nitroalkene thus obtained in the invented process afford better yield and quality of product without purification.

Example 3 constitutes preparation of cyclopropane ring from nitroalkene and sulphur ylide. In this method, to a solution of 4-methyl-1-nitropentene in dry CH_2Cl_2 is added a freshly prepared solution of EDSA, ethyl(dimethylsulphuralidene)acetate in dry CH_2Cl_2 and the mixture is refluxed for six hours. After removal of solvent, residue is purified by column chromatography using silica gel and Petroleum ether as eluent to give ethyl-2-isobutyl-3-nitrocyclopropane carboxylate as a liquid. This invention provides new route for preparation of racemic pregabalin through hydrolysis of cyclopropane ester (example 4) of structure IIIa to compound of structure IIIb followed by reduction (example 5).

Present invention also discloses preparation of racemic pregabalin through novel intermediate of structure IIIc. The process involve hydrolysis of nitrile to corresponding amide (example 6) and then amide to corresponding acid (example 7) followed by reduction with reducing agents. Or other way is direct hydrolysis of nitrile to acid followed by reduction.

The invention, discloses a process for the preparation of pure (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid from its ester via enzymatic kinetic resolution. The method includes preparation of pure (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid with an enantiomeric excess exceeding 99 %. This invention also includes obtaining the (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid with an enantiomeric excess exceeding 98 %. In one embodiment of this invention, enzymes in general and esterases, lipases and proteases in particular are used to achieve enantioselective preparation of pregabalin. Another embodiment of this invention includes use of such enzymes in free form and all modified forms including immobilized preparations. A further embodiment of this invention includes the use of such enzymes in water as well as in co-solvent mixtures with organic solvents and ionic liquids. Yet another embodiment of this invention includes the use of such enzymes in organic solvents, ionic liquids, biphasic mixtures thereof and reverse micellar media.

This invention also includes the approaches outlined in Scheme A and Scheme B. The option would depend upon the stereoselectivity of the enzyme chosen for the kinetic resolution purposes.

The invention includes racemization of (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid ester and or (*R*)-(-)-3-aminomethyl-5-methylhexanoic acid to convert total starting material to the (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid by repeating either Scheme A or Scheme B.

Example 1: Preparation of 4-methyl-1-nitropent-1-ene

To 200 ml of water 7.02 gm (175.5 mmol) of sodium hydroxide was added . To this vigorously stirred solution at 0-5 degree C was added mixture of 15 gm (174.2 mmoles) of isovaleraldehyde and 10.6 gm (174.2 mmoles) of nitromethane drop wise for 1 hour maintaining internal temperature between 0-5 degree C . Soon solid separate out. After 1 hour, 50 ml of excess of ethanol was added to make a slurry, the resultant slurry was filtered and the cake obtained was then neutralized by using 10 ml of conc. HCl and extracted with 2x100 ml of chloroform or dichloromethane . The organic layer was then washed with water, brine and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to give crude product. 4-methyl-1-nitropent1-ene is isolated in 11 gm (50.2%) yield by using column chromatography using elution phase Cyclohexane : ethyl acetate (9:1) comprising preparing a column from slurry of silica gel and cyclohexane, loading the crude compound 4-methyl-1-nitropeta-1-ene on silica gel column, starting the elution with cyclohexane as 100%, slowly raising the eluent phase to 5% ethyl acetate and then to 10%, collecting the fractions, and evaporating the pure fraction/s under vacuum to get pure 4-methyl-1-nitropenta-1-ene. ^{1}H NMR (60 MHz, $CDCl_3$) δ = 0.92-0.98 (d, 6H), 1.82 (m, 1H), 2.13-2.15(m, 2H) , 6.95-7.23 (m, 2H) ppm. ^{13}C NMR(125 MHz, $CDCl_3$) δ = 141.5, 140.1, 37.3, 29.7, 27.8, 22.1 ppm. IR (Neat): 1350, 1554, 1635 cm^{-1}.

Example 1a : Preparation of 4-methyl-1-nitropentan-2-ol

To 200 ml of ethanol or methanol 6.97 gm of sodium hydroxide was added slowly . To this vigorously stirred solution at 0-5 degree C was added 15 gm (0.174 moles) of isovaleraldehyde and 10.6 gm (0.174 moles) of nitromethane drop wise for 1 hour maintaining internal temperature between 0-5 degree C . Soon solid separate out. After 1 hour, 50 ml of excess of ethanol or methanol was added to make a slurry, the resultant slurry was filtered and the cake obtained was then neutralized by using 10 ml of conc. HCl and extracted with 2x100 ml of chloroform. The organic layer was then washed with water, brine and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure to give crude product. 4-methyl-1-nitropentan-2-ol is isolated in 87% yield by using column chromatography using elution phase Cyclohexane : ethyl acetate (8:2)

Example 1b. Conversion of 4-methyl-1-nitropentan-2-ol to 3-methyl-1-(nitromethyl)butylacetate

To 20 gm (135.1 mmol) of 4-methyl-1-nitropentan-2-ol in 150 ml of dichloromethane was added 7.16 gm (67.5 mmole) sodium carbonate. To this vigorously stirred solution 15.91 gm (202.7 mmole) of acetyl chloride was added drop wise over the period of 1.5 hour. Further reaction mass was allowed to stir at 25-27 degree C for 5-6 hours. At this point reaction was monitored by GC and IR. After completion of reaction, mass was quenched by 25 % ammonia solution. The organic layer was then washed with water. Evaporation of dichloromethane under vacuum yields 20.80 gm (82%) of 3-methyl-1-(nitromethyl)butylacetate

IR : 3462 cm^{-1} peak disappear with appearance of peak at 1747 cm^{-1}

Example 1c. Conversion of 3-methyl-1-(nitromethyl)butyl acetate to 4-methyl-1-nitropet-1-ene

To 20 gm (107 mmol) of 3-methyl-1-(nitromethyl)butylacetate in 150 ml of acetone, was added 8.77gm (107 mmole) of sodium acetate. The resultant reaction mixture was refluxed for 3 hour. Reaction was monitored by GC and TLC. After completion of reaction, mass was filtered and solvent was evaporated under vacuum. The residue was then diluted with 500 ml of dichloromethane and washed with 200x2 ml of water to remove acetic acid. The organic layer was then evaporated to yield 10.46 gm (76%) of 4-methyl-1-nitropet-1-ene.

IR : 1747 cm $^{-1}$ peak disaapear with appearance of peak at 1635 cm $^{-1}$

Example 2 : Preparation of Ethyl (Dimethylsulphuranylidene) acetate :

A. Carboethoxymethyl dimethylsulphonium bromide:

A solution of ethyl bromoacetate (20 gm, 119 mmoles) and dimehtyl sulphide (7.44 gm, 121 mmoles) was stirred at room temperature for 72 hours with or without solvent using. White salt was diluted with 50 ml of chloroform and collected by filtration. Sulphonium bromide salt was obtained quantitatively as a white crystalline solid in 21.8 gm 89% yield.

B. Ethyl (dimehtylsulphuranylidene) acetate :

A solution of sulphonium bromide (15 gm, 65.5 mmole) in chloroform (100 ml) was vigorously stirred at 5-10 °C with ice cooling and treated in one portion with mixture of saturated potassium carbonate solution (40 ml) and 12.5 N sodium hydroxide solution (5.20 ml). The reaction mixture was warmed to 15-20 °C and was held for 15 min. After removal of salt by filtration, the filtrate was separated and the upper layer of chloroform was dried for 2 hrs. over anhydrous potassium carbonate. Removal of solvent under vacuum at 25 °C gave the product 7.8 gm as a pale yellow oil in 81%

Saturated potassium carbonate solution was prepared by dissolving 130 gm of K2CO3 in 100 ml of water stirring for 2 hour. As sulfur methylide degrades at room temperature, it was totally used for next reaction.

Example 3: Preparation ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate

To a solution of 4-methyl-1-nitropentene (6.45 gm, 50 mmol) in dry CH_2Cl_2 (50 ml) was added a freshly prepared solution of EDSA, ethyl(dimethylsulphuralidene)acetate. (7.8 gm, 52.7 mmol) in dry CH_2Cl_2 and the mixture was refluxed for six hours. After removal of solvent, residue was purified by column chromatography to give ethyl-2-isobutyl-3-nitrocyclopropane carboxylate as a liquid 5.3 gm (50%) yield.

15 gm Of crude ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate cyclopropane carboxylate were adsorbed on of silica gel (Mesh size 60-120), the said column was prepared by preparing the slurry of silica gel and petroleum ether, the compound was loaded on silica gel column, elution started with petroleum ether as 100%, after three bed volume fractions were collected, the pure fractions were combined and solvent was evaporated under vacuum ^{1}HNMR (300 MHz, $CDCl_3$) δ = 0.90-0.98 (m, 9H), 1.26-1.31 (t, 3H), 1.57-1.71 (m, 2H), 2.13-2.18 (t, 1H), 2.86-2.91 (dd, 1H), 4.15-4.23 (q, 2H), 4.52-4.55(t, 1H) ppm.^{13}C NMR(125 MHz, $CDCl_3$) δ = 14.1 22.0, 22.1, 28.0, 29.9, 30.4, 33.1, 61.4, 63.5, 63.9, 167.3 ppm. IR: 1028, 1186, 1368, 1547, 1759 cm^{-1}

Example 4: Preparation of 2-isobutyl-3-nitrocyclopropane-1-carboxylic acid

5 gm (23.2 mmol) of ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate was added to a solution of 1 mol equivalent of NaOH (0.93 gm) in methanol (40 ml). The reaction mixture was allowed to stir for additional 4 hour at room temperature. After completion of reaction, the mixture was neutralized by conc. HCl and extracted with Chloroform (2 x 50 ml). The organic layer was washed with water, brine and dried over anhydrous sodium sulphate. After removal of solvent under reduced pressure, sticky mass was obtained. After purification by column chromatography, pure acid was obtained in 3.26 gm 75% yield.

Result : white solid, m.p. : 108-110 °C, ^{1}HNMR (300 MHz, CDCl3) δ = 0.95-0.98 (d, 6H), 1.56-1.70 (m, 3H), 2.45(m, 1H), 2.90-2.94 (t, 1H), 4.56-4.622 (t, 1H), 9.816 (bs, 1H) ppm.^{13}C NMR(125 MHz, CDCl3) δ = 22.0, 22.2, 28.0, 29.5, 31.1, 33.1, 64.2, 173.9 ppm. IR: 951, 1220, 1371, 1546, 1693 cm^{-1}

Example 5: Reductive ring opening of 2-isobutyl-3-nitrocyclopropane-1-carboxylic acid

To a solution of Raney Ni (Kaloat 8030) in methanol (50ml) was added 2 gm (10.6 mmol) of cyclopropane carboxylic acid and the reaction was allowed to proceed for 5 hour under hydrogen pressure. Reaction was monitored by TLC. After completion of reaction, the mixture was filtered. Methanol was removed under vacuum to give residue. which is washed with 10ml ethyl acetate to give white solid in 0.500 gm (30% yield).

Results : White solid.

NMR (300 MHz) :

IR (KBr) : u_{max} = 2930, 2697, 2192, 1645, 1550 cm^{-1}.

Example 6: Hydrolysis of nitrile to amide

In 100 ml 15% methanolic HCl 5 gm of nitrile of structure IIIC was dissolved and set to warm for 4 hours. After completion of reaction reactions mass was quenches with methnaolic ammonia. The solid precipitated was then filtered and acid was isolated by evaporation of methanol under vacuum in 4.1 gm (78%) yield.

Example 7 Hydrolysis of amide to carboxylic acid

In 100 ml water 5 gm of amide was suspended and set to reflux in presence of 10ml of sulphuric acid for 8 hours. After completion of reaction reactions mass was quenches with methnaolic ammonia. The solid precipitated was then filtered and acid was isolated by evaporation of methanol under vacuum in 5. 08 gm (87%) yield.

Example 8 : Hydrolysis of nitrile to acid

In 100 ml 30% HCl , 5 gm of nitrile of structure IIIc was suspended and set to reflux for 4 hours. After completion of reaction reactions mass was quenches with methnaolic ammonia. The solid precipitated was then filtered and acid was isolated by evaporation of methanol under vacuum in 1.2 gm (21%) yield.

Example 9 : ESTERIFICATION OF RACEMIC PREGABALIN

Example 9 A : In 500 ml round bottom flask 5 gm of Racemic pregabalin acid was dissolved in 300 ml of methanol . Dry HCl gas was passed through this solution at 5-10 degree C (Absorption of HCl in methanol must be minimum 10 gm) . The whole reaction muixture was allowed to stir at room temperature for 5 hours. After completion of reaction, mass was quenched with methanolic ammonia maintaining pH about 5. Solvent was evaporated under high vacuum. the solid obtained was then filtered by diluting the mixture with DCM (dichloromethane) (150 ml). DCM was evaporated under vacuum to get 4 gm of pregabalin methyl ester.

Example 9 B : In 500 ml round bottom flask 5 gm of Racemic pregabalin acid was dissolved in 300 ml of methanol and 5 ml of sulphuric acid . The whole reaction mixture was allowed to stir at room temperature for 5 hours at 60 degree C. After completion of reaction, mass was quenched with methanolic ammonia maintaining pH about 5. Solvent was evaporated under high vacuum. the solid obtained was then filtered by diluting the mixture with DCM (150 ml). DCM was evaporated under vacuum to get 2 gm of pregabalin methyl ester.

Example 10: ENZYMATIC HYDROLYSIS BY LIPOZYME®RM IM (*Rhizomucor miehei* LIPASE IMMOBILIZED ON ANION EXCHANGE RESIN, FROM NOVOZYME):

In a 250 ml reactor, 1 gm racemic 3-aminomethyl-5-methylhexanoic acid methyl ester (I, Scheme A) was dissolved in 95 ml aqueous sodium phosphate buffer (50 mM, pH= 7) containing varying amount (0.1 %-40 %, v/v) of a water miscible organic solvent acetonitrile, so that the total volume of the reaction mixture was 100 ml. Lipozyme (100 mg, ~10 % enzyme load (active units ~20,000 U/g) and shaken for a time period of 15 h at temperatures within the range of 20 °C -60 °C most preferably at 45-50 degree C. Conversion obtained was 49% . After ~50% conversion reaction mass was quenched and extracted with dichloromethane (DCM). Acid R-isomer was isolated in a yield of about 0.450 gm with optical purity of 90% by evaporation of water layer and 'S' ester was obtained in 0.487 gm by evaporating the DCM layer, Further 'S' ester was subjected for chemical hydrolysis to get S-pregabalin in 0.39 gm of optical purity 94%.

Example 11: ENZYMATIC HYDROLYSIS BY PROTEASE PAPAIN (SIGMA ALDRICH)

In a 250 ml reactor, 1 gm racemic 3-aminomethyl-5-methylhexanoic acid methyl ester (I, Scheme A) was dissolved in 95 ml aqueous sodium phosphate buffer (50 mM, pH= 7.8) containing varying amount (0.1 %-40 %, v/v) of a water miscible organic solvent acetonitrile,. so that the total volume of the reaction mixture was 100 ml. Protease (100 mg) (active units 1.5-10 U/mg) was added and the mixture was shaken at temperatures within the range of 20 °C-60 °C for a time period ranging from 10-72 h . After 72 hours conversion obtained was 51% After ~51% conversion reaction mass was quenched and extracted with DCM. Yield of acid R-isomer acid was obtained in 0.492 gm with optical purity of 97 % by evaporating water layer and S-ester is isolated in a yield of about 0.479 gm by extraction evaporating the DCM layer. Further 'S' ester was subjected for chemical hydrolysis to get S-pregabalin in 0.402gm of optical purity >97%.

Example 12: ENZYMATIC HYDROLYSIS BY SUBTILISIN CARLSBERG (FROM *Bacillus licheniformis*) PROTEASE (SIGMA, USA):

In a 250 ml reactor, 1 gm racemic 3-aminomethyl-5-methylhexanoic acid methyl ester (I, Scheme A) was dissolved in 95 ml aqueous sodium phosphate buffer (50 mM, pH= 7.8) containing varying amount (0.1 %-40 %, v/v) of water miscible organic solvents acetonitrile, N. so that the total volume of the reaction mixture was 100 ml. Subtilisin protease (100 mg) (active units 7-15 U/mg) was added and shaken at temperatures within the range of 20 °C-60 °C for a time period ranging from 2 h-48 h. After 48 hour reaction mass was quenched and extracted with dichloromethane to recover ester. The acid R-isomer was obtained in 0.486 gm after evaporation of water with optical purity of >99%. Ester of S-isomer was obtained in 0.500 gm and subjected for chemical hydrolysis to yield 0.418 gm of S-pregabalin of optical purity > 99%

METHODS USED IN CHEMICAL HYDROLYSIS, RECOVERY OF ESTER AND ANALYSIS

High performance liquid chromatography: The reaction aliquots (20 μl) were diluted by acetonitrile (2X) to precipitate the soluble enzyme out of the medium aided by centrifugation. The supernatant was analyzed in a Zorbax C-18 column by a mixture of acetonitrile and water (30:70, v/v) using UV-DAD at 210 nm with a flow rate of 1ml /min (Ref. van Langen, LM *et al. FEBS Lett*, 1999, 456, 89-92; Lovdahl, MJ *et al.*, *J. Pharm. Biomed. Anal.* 2002,28,917-24).

Recovery of the unreacted alkyl ester : (IIa, Scheme A) is achieved by method described in the following. Near or at 50 % conversion the reactions were quenched by adding methanol (2x dilution) causing the soluble enzyme to precipitate out (in case of Lipozyme, the removal of enzyme was done by filtration and the subsequent work up to evaporate methanol was not required). The supernatant was collected after centrifugation and the methanol was evaporated under strong vacuum and finally the ester was extracted by dichloromethane (Ref. Chen ST *et al.*, *J.Chem.Soc.,Chem. Commun.*, 1986, 606, 1514-16), dichloromethane (1 ml, 0.3 ml each time, thrice for a 1ml aqueous solution). The ester was recovered after the evaporation of the dicholoromethane layer and weighed. Yield~98-99 % as compared to the expected value.

Hydrolysis of the alkyl ester: The alkyl ester (0.500 gm) was chemically hydrolyzed by 50 ml 2N hydrochloric acid for 5 h at 30 °C. The excess acid was neutralized by 0.6 N aquec bonate solution. The evaporation of aqueous layer gav which was dissolved in methanol and filtered. The evaporation of methanol gave the S-isomer of pregabalin from 0.389-0.415 gm yield

Melting point : 183-185 °C

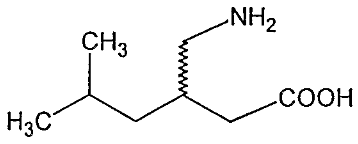
IR: 1552, 1637, 2210, 2570, 2645, 2937, 3420 Cm^{-1}

NMR : 0.74-0.77 (m, 6H), 1.05-1.10 (t, 2H), 1.45-1.56 (m, 1H), 1.98-2.218(m, 3H), 2.79-2.88 (d,2H)

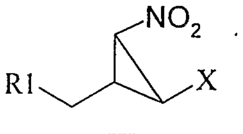
Polarimetric analysis: From the conversion values directly obtained by HPLC, the concentrations of the hydrolysed pregabalin, unreacted methyl ester and hence that of the chemically hydrolyzed pregabalin from the unreacted ester were determined. The specific rotations were obtained from these concentration values after analysis in Rudolph ®Autopol V polarimeter in a 10 mm cell (0.23 ml). From these values enantiomeric excess were calculated. $[\alpha]_D = +10^\circ$ (concentration = 0.5 %, in water at 32° C) for optically pure (*S*)-(+)-3-aminomethyl-5-methylhexanoic acid.

CLAIMS

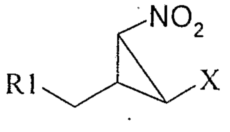
1. A process of preparation of a compound of structure I



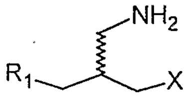
I from a precursor compound of structure III



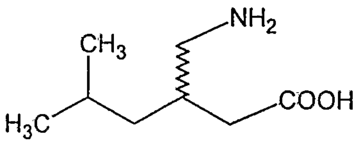
III wherein, R1 = Alkyl, Aryl, allyl, heterocycle, straight chain or branch chain, X=-CN or -COOR, wherein R may be hydrogen, or straight chain alkyl group or branch chain alkyl group; the said process comprising reductive ring opening of compound of structure



III to form compound of structure



IV IV that results in racemic pregabalin of structure I in a situation (a) when X = -COOH , and R1 = isopropyl group, or (b) When X = -CN, and R1 is isopropyl group, then either by (i) conversion of -CN to –C(O)NH2, nitrile to amide, followed by hydrolysis of the amide group to carboxylic acid group and reduction, or by (ii) direct conversion of nitrile to acid; the said racemic pregabalin of structure I being optionally resolved to get substantially pure (S) pregabalin of structure I



I

2. A process of claim 1 wherein the said reductive ring opening is achieved by reacting the compound of structure III with a reducing agent in a polar solvent at a temperature and for a period of time enough for effecting the said ring opening.

3. A process of claim 2 wherein:

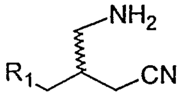
    a. the said reducing agent comprises Raney Ni/ H_2 or Pd/C or Zn/$HCOONH_4$ or Zn/HCOOH or Fe/CH_3COOH or Fe/HCl.

    b. the said temperature is about 25 °C, and

    c. the said polar solvent comprises methanol or water or combination of both,

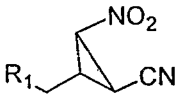
    d. the said period is about 5 hours.

4. A process of claim 2 wherein to a solution of Raney Ni (Kaloat 8030) in methanol is added 3-isobutyl-2-nitrocyclopropane-1-carboxylic acid and the reaction is allowed to proceed for about 5 hour under hydrogen pressure until completion, the mixture is filtered, methanol is removed under vacuum to give residue of racemic pregabalin of structure I if X=COOH or of a structure IVa if X= -CN



IVa

5. A process of claim 1 comprising conversion of structure IIIc

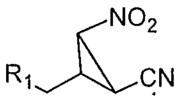
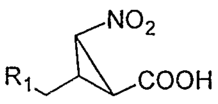


IIIc to amide and further the amide group is hydrolysed to carboxylic acid followed by reduction of nitro to amine

6. A process of claim 5 comprising dissolving compound of structure IIIc in methanolic HCl and setting to warm for 4 hours, after completion of reaction reactions, the mass is quenched with methnaolic ammonia, the solid precipitated,

is filtered and acid is isolated by evaporation of methanol under vacuum, hydrolyzing the amide obtained by using sulphuric acid under reflux for about 8 hours, quenching the reaction after completion by methanolic ammonias to precipitate the carboxylic acid and its isolation by removal of methanol by evaporation under vacuum.

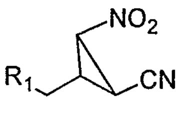
7. A process of claim 1 of directly converting compound of structure

IIIc IIIc to a compound of structure IIIa IIIa comprising of adding nitrile of structure IIIc to hydrochloric acid , refluxing the mixture for appropriate time, quenching of reaction mass with methnaolic ammonia. filtration of precipitated solid and evaporation of methanol under vacuum.

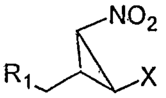
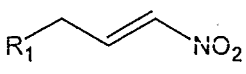
8. A process of claim 7 comprising adding amide dissolved in water and set to reflux in presence of sulphuric acid for 8 hours, after completion of reaction, the reaction mass was quenched with methnaolic ammonia, the solid precipitated was then filtered and acid was isolated by evaporation of methanol under vacuum.

9. A compound of structure IIIc



IIIc , where R1 R1=Alkyl, Aryl, allyl, heterocycle, straight chain or branch chain.

10. A process of claim 1 wherein preparation of compound of structure III

III by reacting a compound of structure VII VII with a sulphur ylide or phoshphorus ylide of formula R_2 YCH_2 at reflux temperature in chlorinated solvent, wherein R_2 = Alkyl, aryl, Y = P or S and X=-CN or -COOR, wherein R may be hydrogen, or a straight chain alkyl group or a branch chain alkyl group.

11. A process of claim 10 comprising following steps:

   a. adding a freshly prepared solution of ethyl(dimethylsulphuralidene)acetate of structure $(CH_3)_2S{=}CHX$ made in dry CH_2Cl_2 to a solution of 4-methyl-1-nitropentene in dry CH_2Cl_2,

   b. refluxing the mixture for about six hours,

   c. removal of solvent,

   d. getting the residue of ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate.

12. A process of claim 11 comprising purifying the said residue by column chromatography using Petroleum ether as solvent to give substantially purified ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate cyclopropane carboxylate as a liquid.

13. A process of claim 12 comprising

Adsorbing crude ethyl-2-isobutyl-3-nitrocyclopropanecarboxylate cyclopropane carboxylate on of silica gel (Mesh size 60-120), the said column being prepared by preparing the slurry of silica gel and petroleum ether, compound is loaded on silica gel column, elution started with petroleum ether as 100%, after three bed volume fractions were collected, the pure fractions were combined and solvent was evaporated under vacuum.

14. A process of claim 10 where the compound of structure VII is prepared by reacting isovaleraldehyde with nitromethane under basic conditions accompanied with stirring for a period sufficient for formation of a compound of structure VII and a compound of structure V.

15. A process of claim 14 comprising basic conditions are created by using 50% aqueous NaOH or a 50% NaOH in ethanol, or a 50% NaOH in methanol and period for reaction between isovaleraldehyde and nitromethane is about three hour.

16. A process of claim 14 comprising following steps:

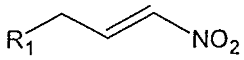
    a. Adding sodium hydroxide to water,
    b. to this vigorously stirred solution, at about 0-5° C was added mixture of isovaleraldehyde and nitromethane drop wise for a period of about 1 hour maintaining internal temperature between about 0-5 degree C achieving separation of solids,
    c. after 1 hour, excess of ethanol is added to make a slurry,
    d. the resultant slurry is filtered,
    e. the cake obtained is then neutralized by concentrated HCl and extracted with chloroform or dichloromethane.
    f. the organic layer is then washed with water, brine and dried over anhydrous sodium sulphate,
    g. the solvent was evaporated under reduced pressure to give crude product. 4-methyl-1-nitropent1-ene and 4-methyl-1-nitropentane-2-ol
    h. 4-methyl-1-nitropent-1-ene is isolated and purified.

17. A process of claim 16 comprising isolation and purification of 4-methyl-1-nitropent1-ene by using column chromatography using elution phase Cyclohexane : ethyl acetate (9:1).

18. A process of claim 17 comprising following steps: preparing a slurry of silica gel and cyclohexane, loading the crude compound 4-methyl-1-nitropeta-1-ene on

silica gel column, starting the elution with cyclohexane as 100%, slowly raising the eluent phase to 5% ethyl acetate and then to 10%, collecting the fractions, and evaporating the pure fraction/s under vacuum to get pure 4-methyl-1-nitropenta-1-ene.

19. A process of producing a compound of structure VII



VII

comprising acylation of compound of structure V followed by elimination.

20. A process of claim 19 comprising use of acyl chloride or acyl anhydride as the acylating reagent in a chlorinated solvent with a base in a dry solvent.

21. A process of claim 20 comprising:

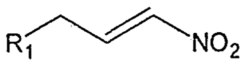
a. the said acyl chloride preferably being acetyl chloride,

b. the said chlorinated solvent including one or more of a chloroform, dichloromethane or ethylene dichloride or a mixture thereof,

c. the said base selected for elimination comprises anhydrous sodium acetate or sodium carbonate,

d. and the said dry solvent includes THF or acetone.

22. A process of producing a compound of structure VII



VII

comprising following steps:

a. reacting isovaleraldehyde with nitromethane under conditions suitable for preparing 4-methyl-1-nitropentan-2-ol .

b. conversion of 4-methyl-1-nitropentan-2-ol under suitable conditions to 3-methyl-1-(nitromethyl)butylacetate, and

c. conversion of 3-methyl-1-(nitromethyl)butyl acetate under suitable conditions to 4-methyl-1-nitropet-1-ene.

23. A process of claim 22 of preparing 4-methyl-1-nitropentan-2-ol comprising following steps:

a. to ethanol or methanol, sodium hydroxide iss added slowly,

b. to this vigorously stirred solution at about 0-5° C is added isovaleraldehyde and nitromethane drop wise over about 1 hour maintaining internal temperature between about 0-5 degree C,

c. Getting separation of solids,

d. after 1 hour, adding an excess of ethanol or methanol was added to make a slurry,

e. filtering the resultant slurry,

f. neutralizing the cake obtained was by using concentrated HCl and extracting with chloroform,

g. washing the organic layer with water, brine,

h. drying over anhydrous sodium sulphate,

i. evaporating the solvent under reduced pressure to get crude product 4-methyl-1-nitropentan-2-ol.

24. A process of claim 23 comprising purification and isolation of 4-methyl-1-nitropentan-2-ol by using column chromatography.

25. A process of claim 23 comprising following steps: preparing the column with slurry of silica gel and cyclohexane, loading the crude compound of 4-methyl-1-nitropentan-2-ol on column, eluting with cyclohexane as 100% and then slowly raising to combination of 90:10% of ethyl acetate and then to 20%, and eluting

with 10% to remove compound of structure VII, and elution with 20% ethyl acetate to getpure 4-methyl-1-nitropentane-2-ol.

26. A process of claim 23 comprising adding reactants in following proportions and in the conditions of reaction as follows: add 6.97 gm of sodium hydroxide slowly to 200 ml of ethanol or methanol, stir the solution vigorously at 0-5° C and add 15 gm (0.174 moles) of isovaleraldehyde and 10.6 gm (0.174 moles) of nitromethane drop wise for 1 hour maintaining internal temperature between 0 - 5 ° C, one hour after the solid separate out, 50 ml of excess of ethanol or methanol is added to make a slurry, the resultant slurry is filtered and the cake obtained is then neutralized by using 10 ml of conc. HCl and extracted with 2x100 ml of chloroform, the organic layer is then washed with water, brine and dried over anhydrous sodium sulphate, the solvent is evaporated under reduced pressure to give crude product 4-methyl-1-nitropentan-2-ol which is isolated and purified by using column chromatography using elution phase cyclohexane : ethyl acetate (8:2).

27. A process of claim 22 of conversion of 4-methyl-1-nitropentan-2-ol to 3-methyl-1-(nitromethyl)butylacetate comprising following steps:

    a. to a solution of 4-methyl-1-nitropentan-2-ol in dichloromethane is added solid sodium carbonate,

    b. to this vigorously stirred solution, acetyl chloride is added drop wise over about a period of 1.5 hour,

    c. the reaction mass is allowed to stir at about 25-27° C for about 5-6 hours,

    d. after completion of reaction, the mass was quenched by about 25 % ammonia solution,

    e. the organic layer was then washed with water,

f. dichloromethane was evaporated under vacuum to yield 3-methyl-1-(nitromethyl)butyiacetate.

28. A process of claim 27 comprising adding reactants in following proportiions and in the conditions of reaction as follows: to 20 gm (135.1 mmol) of 4-methyl-1-nitropentan-2-ol in 150 ml of dichloromethane is added 7.16 gm (67.5 mmole) sodium carbonate, to this vigorously stirred solution 15.91 gm (202.7 mmole) of acetyl chloride is added drop wise over the period of 1.5 hour, the reaction mass was allowed to stir at 25-27 degree C for 5-6 hours, after completion of reaction, mass was quenched by 25 % ammonia solution, the organic layer is then washed with water, dichloromethane is evaporated under vacuum to get 3-methyl-1-(nitromethyl)butylacetate.

29. A process of claim 22 comprising conversion of 3-methyl-1-(nitromethyl)butyl acetate to 4-methyl-1-nitropet-1-ene comprising steps of:

a. to a solution of 3-methyl-1-(nitromethyl)butylacetate in acetone, sodium acetate is added,

b. the resultant reaction mixture is refluxed for about 3 hour,

c. after completion of reaction, mass is filtered and solvent is evaporated under vacuum,

d. the residue is then diluted with dichloromethane,

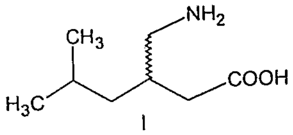
e. washed with water to remove acetic acid,

f. evaporating the organic layer was to yield 4-methyl-1-nitropet-1-ene.

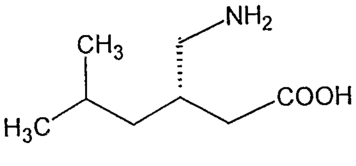
30. A process of claim 29 comprising adding reactants in following proportiions and in the conditions of reaction as follows: to 20 gm (107 mmol) of 3-methyl-1-nitromethyl)butylacetate in 150 ml of acetone, is added 8.77gm (107 mmole) of sodium acetate, the resultant reaction mixture is refluxed for 3 hour, after completion of reaction, mass is filtered and solvent is evaporated under vacuum,

the residue is then diluted with 500 ml of dichloromethane, washed with 200x2 ml of water to remove acetic acid, the organic layer is then evaporated to yield 4-methyl-1-nitropet-1-ene.

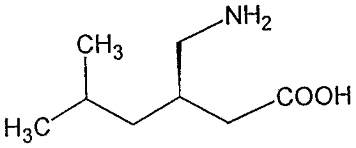
1. A process of resolution of racemic pregabalin esters of structure I



I into (S) pregabalin of structure Ia



Ia and (R) pregabalin of structure



Ib comprising:

a. treating a solution of esters prepared from racemic pregabalin by an enzyme under appropriate conditions capable of converting one of the enantiomer ester into corresponding acid enantiomer in a substantially good yield resulting into a mixture of an ester, that has not been acted upon by the enzyme used, of one enantiomer of pregabalin and acid of the another enantiomer that is formed by the enzyme action,

b. separating the esters, comprising one enantiomer ester that has remained unchanged by the enzyme action and the other enantiomer of pregabalin formed by enzyme action on the corresponding enantiomer of pregbalin ester, by an appropriate process of separation,

c. collecting the acid enantiomer saparated ,

d. optionally hydrolyzing the separated ester enantiomer into corresponding pregabalin enantiomer by hydrolysis.

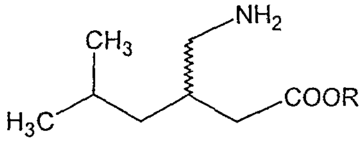
32. A process of claim 31 comprising hydrolysis of the ester by (a) chemical hydrolysis, or (b) by an enzyme in one or more of an organic solvent, an ionic liquid, a biphasic mixture thereof and a reverse micellar medium.

33. A process of claim 31 wherein the (a) chemical hydrolysis is either an acid hydrolysis or an alkaline hydrolysis, and (b) the enzymatic hydrolysis, is by an esterase.

34. A process of claim 33 comprising an enzymatic hydrolysis comprising selection of an esterase, or a hydrolase selected from at least a lipase, a protease, an esterase, a hydrolase or an alkalase.

35. A process of claim 34 comprising following steps:

  a. racemic 3-aminomethyl-5-methylhexanoic acid methyl ester of structure



II is dissolved in aqueous sodium phosphate buffer (about 50 mM, about pH = 7) containing varying amount of at least one water miscible organic solvent,

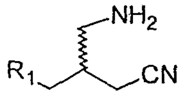
  b. the enzyme is added and shaken for a time period and at a temperatures range that is appropriate for conversion of substantial portion of R- 3-aminomethyl-5-methylhexanoic acid methyl ester to 3-aminomethyl-5-methylhexanoic acid.

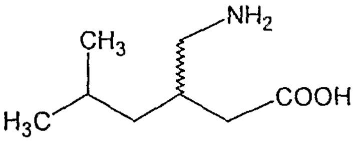
36. A process of claim 35 wherein:

  a. water miscible organic solvent varied between 0.1 % - 40 %, v/v, and included at least an acetonitrile, N,N-dimethyl formamide, dimethyl sulphoxide, or tetrahydrofuran,

  b. time period of shaking is about 6 -18 h,

c. temperature is kept within the range of about 20 °C - 60 °C.


IVa


I

37. A process of conversion of a compound of structure IVa to racemic pregabalin of structure I by enzymatic conversion of _-CN group at first carbon atom to the action of an enzyme that is capable of regioselectively as well as sterioselectively convertion of nitrile group to carboxyl group by enzymatic action of an enzyme capable of stereoselctive conversion of the compound of structure IV to (S)-(+) pregabalin as the only isomer.

38. A process of claim 37 comprising the use of an enzyme in one or more of an organic solvent, an ionic liquid, a biphasic mixture thereof and a reverse micellar medium.

39. A process of claim 37 comprising following steps:

a. A compound of structure IVa as given above is dissolved in aqueous sodium phosphate buffer (about 50 mM, about pH = 7) containing varying amount of at least one water miscible organic solvent,

b. the enzyme capable of stereoselctive conversion to (S) pregabalin is added and shaken for a time period and at a temperatures range that is appropriate for conversion of substantial portion of R- 3-aminomethyl-5-methylhexanoic acid methyl ester to 3-aminomethyl-5-methylhexanoic acid.

40. A process of claim 38 wherein:

a. water miscible organic solvent varied between 0.1 % - 40 %, v/v, and included at least an acetonitrile, N,N-dimethyl formamide, dimethyl sulphoxide, or tetrahydrofuran,

b. time period of shaking is about 6 -18 h,

c. temperature is kept within the range of about 20 °C - 60 °C

41. A process of claim 39 comprising use of nitrilase as the enzyme of choice.