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(54) Title: STEREOISOMERICALLY ENRICHED BETA-LACTAMS USING CANDIDA ANTARCTICA

(57) Abstract: The present disclosure provides enzymatic methods for generating stereoisomerically pure products by resolving a racemic mixture of N-protected β -lactams with a lipase from *Candida antarctica* with high stereospecificity. The presence of a carbamate protecting group, such as the tert-butoxycarbonyl group protecting group, on the (3-lactam enhances enzyme catalysis and stereoselectivity.



WO 2006/055528 A2

STEREOMERICALLY ENRICHED β -LACTAMS USING CANDIDA ANTARCTICA

1. CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit under 35 U.S.C. § 119(e) to application Serial No. 60/628,401, filed November 15, 2004, the contents of which is incorporated herein by reference.

2. FIELD

[0002] The present disclosure relates to methods for generating stereospecific products from a racemic mixture of N-protected β -lactams that can be used for chiral syntheses. In particular, the method can be used to synthesize stereoisomerically pure β -lactam and β -amino carboxylic acid compounds that can be used as starting materials to synthesize a wide variety of stereoisomerically pure compounds.

3. BACKGROUND

[0003] The use of enzymes in organic syntheses has become prevalent. By exploiting the tools that nature provides, chiral molecules can be selectively separated or resolved, or chirality can be infused into a molecule having no chirality, by virtue of the enzyme's own chiral nature. Enzymes provide precise stereocontrol and accelerate chemical transformations that are otherwise difficult to carry out using conventional synthetic chemistry. More importantly, enzymes generally obviate the need for protecting group manipulations frequently hampering and adding additional steps in a chemical synthesis. As such, enzymes are commercially used to synthesize and/or resolve active pharmaceuticals and useful intermediates leading thereto.

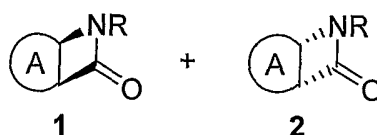
[0004] Many carbon based pharmaceuticals as well as intermediates have chiral centers which permit multiple stereoisomers or antipodes. Typically, each stereoisomer has distinct chemical and physical properties. Some of those properties can be fatal and some can be pharmaceutically useful. For example, (S)-thalidomide can cause severe birth defects, while (R)-thalidomide is a safe and effective sedative, and a treatment for diseases such as cancer. These dramatic differences have led the Food and Drug Administration to require each enantiomer of a racemic drug to be put through clinical testing individually prior to receiving approval for widespread distribution. Thus, there is a need for stereoselective methods for synthesizing or enriching stereoisomers. In

particular, there exists a need for biocatalytic process for synthesizing enantiomeric or diastereomerically pure pharmaceutical and intermediates.

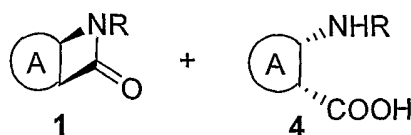
4. SUMMARY

[0005] The present disclosure provides methods for generating stereospecific products from a racemic mixture of N-protected β -lactams that can be used for chiral synthesis. The method can be used to synthesize stereoisomerically pure β -lactam and β -amino carboxylic acid derivatives that can be used as starting materials to synthesize specific diastereomers of a wide variety of molecules. For example, the stereoisomerically pure β -lactam can be used as a starting material to synthesize diastereomerically pure antiproliferative (1R,2R,3S,4S)-N4-(3-aminocarbonylbicyclo[2.2.1]hept-5-ene-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine, and the various compounds described in copending application Serial No. 11/133,419 filed May 18, 2005, international application no. PCT/US05/17470 and application Serial No. _____, entitled "Stereoisomerically Enriched 3-Aminocarbonyl Bicycloheptene Pyrimidinediamine Compounds and Their Uses," filed concurrently herewith (identified by attorney docket no. 375462-039US).

[0006] The method generally comprises contacting a (2-exo, 3-exo) *cis* β -lactam comprising a mixture of enantiomers according to structural formulae 1 and 2:



wherein **A** represents a saturated or unsaturated, monocyclic, polycyclic or bridged polycyclic ring and **R** represents a protecting group, with a lipase from *Candida antarctica* to yield stereoisomerically pure products according to structural formulae 1 and 4:



[0007] Examples of ring **A** include, but are not limited to, bicycloheptenyl, bicycloheptyl, cyclohexenyl, cyclohexenyl, cyclopentyl, cyclopentenyl, cyclopropyl, cyclobutyl, and the like.

[0008] Ring A can be substituted at one or more carbon atoms, or optionally interrupted by, a combination of one or more alkyl, alkanyl, alkenyl, alkynyl, alkylidyl, alkylene, cycloalkyl, aryl, halogen, haloalkyl, hydroxalkyl, thiols, amines, hydroxyls, ethers, alkoxy, C=O, S=O, P=O, nitro, cyano, Se=O and/or N=O groups.

[0009] Suitable protecting groups **R** have the formula -C(X)YR¹, where X is O or S; Y is O or S; and R¹ is selected from unsubstituted or substituted lower alkyl, unsubstituted or substituted lower alkanyl, unsubstituted or substituted (C6-C14) aryl and unsubstituted or substituted (C7-C20) arylalkyl. Thus, protecting group **R**, taken together with the β -lactam nitrogen to which it is bonded, forms a carbamate or a carbamate equivalent, such as a thiocarbamate. In some embodiments, R¹ is selected from *t*-butyl, benzyl and fluoren-9-yl.

[0010] In some embodiments, the β -lactam is protected as a carbamate such that protecting group **R** is of the formula -C(O)OR¹, where R¹ is as previously defined. In some embodiments, R¹ is selected from unsubstituted lower alkyl or alkanyl, unsubstituted or substituted mono-, bi- or tricyclic (C6-C14) aryl and unsubstituted (C7-C20) arylalkyl. Specific, non-limiting examples of exemplary carbamate protecting groups **R** include *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl and 9-fluorenylmethoxycarbonyl.

[0011] Lipases derived from *Candida antarctica* are commercially available in different forms such as resin-immobilized, lyophilized or suspended preparations. All of these lipases can be used in the methods described herein. For ease of handling, immobilized lipases are advantageous. Furthermore, although, resolution of N-protected racemic β -lactam works with stoichiometric or excess amounts of enzyme, only a catalytic amount of the enzyme is needed.

[0012] The resolution of N-protected racemic β -lactam can proceed in various solvents, including but not limited to, diisopropyl ether, butanol, tetrahydrofuran, toluene, hexanes and mixtures of such solvents. Typically, for more efficient conversion, the reaction solvent(s) contains a catalytic amount of water, for example, in the range of about 0.1-1.0%. The necessary amount of water can be achieved by using commercially available anhydrous solvents without further purification or distillation.

[0013] Another variable that affects the enzyme-mediated resolution of racemates is temperature. The temperature can be varied and modulated to accelerate or decelerate the resolution process. In general, the enzymatic resolution of racemates can proceed at a temperature in the range of about 0-80 °C, or more specifically in the range of about 20-60 °C.

5. DETAILED DESCRIPTION

5.1 Definitions

[0014] As used herein, the following terms are intended to have the following meanings:

[0015] "Alkyl" by itself or as part of another substituent refers to a saturated or unsaturated branched, straight chain or cyclic monovalent hydrocarbon radical having the stated number of carbon atoms (i.e., C1-C6 means one to six carbon atoms) that is derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene or alkyne. Typical alkyl groups include, but are not limited to, methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl, prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butyls such as butan-1-yl, butan-2-yl, 2-methylpropan-1-yl, 2-methyl propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like. Where specific levels of saturation are intended, the nomenclature "alkanyl," "alkenyl" and/or "alkynyl" is used, as defined below.

"Lower alkyl" refers to an alkyl group containing from 1 to 6 carbon atoms.

[0016] "Alkanyl" by itself or as part of another substituent refers to a saturated branched, straight chain or cyclic alkyl derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane. Typical alkanyl groups include, but are not limited to, methanyl; ethanyl; propanyls such as propan-1-yl, propan-2-yl (isopropyl), cyclopropan-1-yl, etc.; butanyls such as butan-1-yl, butan-2-yl (*sec* butyl), 2-methyl propan-1-yl (isobutyl), 2-methyl propan-2-yl (*t*-butyl), cyclobutan-1-yl, etc.; and the like.

[0017] "Alkenyl" by itself or as part of another substituent refers to an unsaturated branched, straight chain or cyclic alkyl having at least one carbon carbon double bond derived by the removal of one hydrogen atom from a single carbon atom of a parent

alkene. The group may be in either the cis or trans conformation about the double bond(s). Typical alkenyl groups include, but are not limited to, ethenyl; propenyls such as prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl, prop-2-en-2-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl; butenyls such as but-1-en-1-yl, but-2-en-1-yl, 2-methyl prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, etc.; and the like.

[0018] "Alkynyl" by itself or as part of another substituent refers to an unsaturated branched, straight chain or cyclic alkyl having at least one carbon carbon triple bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkyne. Typical alkynyl groups include, but are not limited to, ethynyl; propynyls such as prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butynyls such as but-1-yn-1-yl, but-1-yn-3-yl, but 3-yn-1-yl, etc.; and the like.

[0019] "Alkyldiyl" by itself or as part of another substituent refers to a saturated or unsaturated, branched, straight chain or cyclic divalent hydrocarbon group having the stated number of carbon atoms (i.e., C1 C6 means from one to six carbon atoms) derived by the removal of one hydrogen atom from each of two different carbon atoms of a parent alkane, alkene or alkyne, or by the removal of two hydrogen atoms from a single carbon atom of a parent alkane, alkene or alkyne. The two monovalent radical centers or each valency of the divalent radical center can form bonds with the same or different atoms. Typical alkyldiyl groups include, but are not limited to, methandiyl; ethyldiyls such as ethan-1,1-diyl, ethan-1,2-diyl, ethen-1,1-diyl, ethen-1,2-diyl; propyldiyls such as propan-1,1-diyl, propan-1,2-diyl, propan-2,2-diyl, propan-1,3-diyl, cyclopropan-1,1-diyl, cyclopropan-1,2-diyl, prop-1-en-1,1-diyl, prop-1-en-1,2-diyl, prop-2-en-1,2-diyl, prop-1-en-1,3-diyl, cycloprop-1-en-1,2-diyl, cycloprop-2-en-1,2-diyl, cycloprop-2-en-1,1-diyl, prop-1-yn-1,3-diyl, etc.; butyldiyls such as, butan-1,1-diyl, butan-1,2-diyl, butan-1,3-diyl, butan-1,4-diyl, butan-2,2-diyl, 2-methyl propan-1,1-diyl, 2-methyl-propan-1,2-diyl, cyclobutan-1,1-diyl; cyclobutan-1,2-diyl, cyclobutan-1,3-diyl, but-1-en-1,1-diyl, but-1-en-1,2-diyl, but-1-en-1,3-diyl, but-1-en-1,4-diyl, 2-methyl-prop-1-en-1,1-diyl, 2-methanylidene propan-1,1-diyl, buta-1,3-dien-1,1-diyl, buta-1,3-dien-1,2-diyl, buta-1,3-dien-1,3-diyl, buta-1,3-dien-1,4-diyl, cyclobut-1-en-1,2-diyl, cyclobut-1-en-1,3-diyl, cyclobut-2-en-1,2-diyl, cyclobuta-1,3-dien-1,2-diyl, cyclobuta-1,3-dien-1,3-diyl, but-1-

yn-1,3-diyl, but-1-yn-1,4-diyl, buta-1,3-diyn-1,4-diyl, etc.; and the like. Where specific levels of saturation are intended, the nomenclature alkanyldiyl, alkenyldiyl and/or alkynyldiyl is used. Where it is specifically intended that the two valencies be on the same carbon atom, the nomenclature “alkylidene” is used. A “lower alkylidiyl” is an alkylidiyl group containing 1 to 6 carbon atoms. In some embodiments the alkylidiyl groups are saturated acyclic alkanyldiyl groups in which the radical centers are at the terminal carbons, e.g., methandiyl (methano); ethan-1,2-diyl (ethano); propan-1,3-diyl (propano); butan-1,4-diyl (butano); and the like (also referred to as alkenes, defined *infra*).

[0020] “Alkylene” by itself or as part of another substituent refers to a straight chain saturated or unsaturated alkylidiyl group having two terminal monovalent radical centers derived by the removal of one hydrogen atom from each of the two terminal carbon atoms of straight chain parent alkane, alkene or alkyne. The locant of a double bond or triple bond, if present, in a particular alkylene is indicated in square brackets. Typical alkylene groups include, but are not limited to, methylene (methano); ethylenes such as ethano, etheno, ethyno; propylenes such as propano, prop[1]eno, propa[1,2]dieno, prop[1]yno, etc.; butylenes such as butano, but[1]eno, but[2]eno, buta[1,3]dieno, but[1]yno, but[2]yno, buta[1,3]diyno, etc.; and the like. Where specific levels of saturation are intended, the nomenclature alkano, alkeno and/or alkyno is used. In some embodiments, the alkylene group is (C1-C6) or (C1-C3) alkylene. In some embodiments, the alkylene group is a straight chain saturated alkano group, e.g., methano, ethano, propano, butano, and the like.

[0021] “Cycloalkyl” by itself or as part of another substituent refers to a cyclic version of an “alkyl” group. Typical cycloalkyl groups include, but are not limited to, cyclopropyl; cyclobutyls such as cyclobutanyl and cyclobutenyl; cycloheptyls such as cycloheptanyl and cycloheptenyl; cyclohexyls such as cyclohexanyl and cyclohexenyl; cycloheptyls such as cycloheptanyl and cycloheptenyl; and the like.

[0022] “Aryl” by itself or as part of another substituent refers to a monovalent aromatic hydrocarbon group having the stated number of carbon atoms (i.e., C6-C14 means from 6 to 14 carbon atoms) derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to,

groups derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, coronene, fluoranthene, fluorene, hexacene, hexaphene, hexalene, as indacene, s indacene, indane, indene, naphthalene, octacene, octaphene, octalene, ovalene, pentacene, pentalene, pentaphene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene, rubicene, triphenylene, trinaphthalene, and the like, as well as the various hydro isomers thereof. In some embodiments, the aryl group is (C6-C10). Specific examples are phenyl and naphthyl.

[0023] "Halogen" or "Halo" by themselves or as part of another substituent, unless otherwise stated, refer to fluoro, chloro, bromo and iodo.

[0024] "Haloalkyl" by itself or as part of another substituent refers to an alkyl group in which one or more of the hydrogen atoms are replaced with a halogen. Thus, the term "haloalkyl" is meant to include monohaloalkyls, dihaloalkyls, trihaloalkyls, etc. up to perhaloalkyls. For example, the expression "(C1-C2) haloalkyl" includes fluoromethyl, difluoromethyl, trifluoromethyl, 1-fluoroethyl, 1,1-difluoroethyl, 1,2-difluoroethyl, 1,1,1-trifluoroethyl, perfluoroethyl, etc.

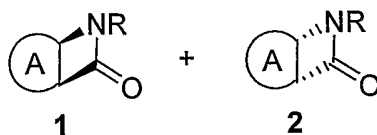
[0025] "Hydroxyalkyl" by itself or as part of another substituent refers to an alkyl group in which one or more of the hydrogen atoms are replaced with a hydroxyl substituent. Thus, the term "hydroxyalkyl" is meant to include monohydroxyalkyls, dihydroxyalkyls, trihydroxyalkyls, etc.

[0026] The above defined groups may include prefixes and/or suffixes that are commonly used in the art to create additional well recognized substituent groups. As examples, "alkyloxy" or "alkoxy" refers to a group of the formula -OR', "alkylamine" refers to a group of the formula -NHR' and "dialkylamine" refers to a group of the formula -NR'R', where each R' is independently an alkyl. As another example, "haloalkoxy" or "haloalkyloxy" refers to a group of the formula -OR'', where R'' is a haloalkyl.

5.2 Resolution of Stereoisomerically Pure β -Lactams Using *Candida Antarctica*

[0027] In one aspect, a method is provided for resolving mixtures of β -lactam diastereomers and/or enantiomers, such as racemic mixtures of (2-exo, 3-exo) *cis* β -lactams, into stereoisomerically pure products using a lipase enzyme from *Candida antarctica*. In some embodiments, the method comprises contacting a (2-exo, 3-exo) *cis*

N-protected β -lactam comprising a mixture of enantiomers according to structural formulae 1 and 2:



with a lipase from *Candida antarctica*, wherein **A** represents a saturated or unsaturated, monocyclic, polycyclic or bridged polycyclic ring and **R** represents a protecting group.

[0028] The **A** ring can be substituted at one or more carbon atoms, or interrupted by, a combination of one or more alkyl, alkanyl, alkenyl, alkynyl, alkylidyl, alkylene, cycloalkyl, aryl, halogen, haloalkyl, hydroxalkyl, thiols, amines, hydroxyls, ethers, alkoxy, C=O, S=O, P=O, nitro, cyano, Se=O and/or N=O groups. Examples of **A** rings include, but are not limited to, bicycloheptenyl, bicycloheptyl, cyclohexyl, cyclohexenyl, cyclopentyl, cyclopentenyl, cyclopropyl, and the like.

[0029] Suitable protecting groups **R** have the formula $-C(X)YR^1$, where, X is O or S; Y is O or S; and R^1 is selected from unsubstituted or substituted lower alkyl, unsubstituted or substituted lower alkanyl, unsubstituted or substituted (C6-C14) aryl and unsubstituted or substituted (C7-C20) arylalkyl. Thus, protecting group **R**, taken together with the β -lactam nitrogen to which it is bonded, forms a carbamate or a carbamate equivalent, such as a thiocarbamate. In some embodiments, R^1 is selected from *t*-butyl, benzyl and fluoren-9-yl.

[0030] In some embodiments, the β -lactam is protected as a carbamate such that protecting group **R** is of the formula $-C(O)OR^1$, where R^1 is as previously defined. In some embodiments, R^1 is selected from unsubstituted lower alkyl or alkanyl, unsubstituted or substituted mono-, bi- or tricyclic (C6-C14) aryl and unsubstituted (C7-C20) arylalkyl. Specific, non-limiting examples of exemplary carbamate protecting groups **R** include *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl and 9-fluorenylmethoxycarbonyl.

[0031] The importance of the carbamate protecting group, or its equivalent, in the enzymatic resolution of racemic β -lactams is apparent from the discussion below.

[0032] In general, lipases are enzymes that catalyze the hydrolysis of fats into fatty acids and glycerol. In addition to resolving esters (see, e.g., Kurokawa et al., 2004, Bull. Chem. Soc. Jpn 77:1021-1025), it has been reported that some lipases from *Candida antarctica* resolve racemic β -lactams that are either unprotected at the β -lactam amide nitrogen atom (via opening of the lactam ring), or that are protected at this nitrogen atom with an N-acyloxymethyl ester protecting group (via hydrolysis of the remote ester group) (see, Forró et al., 2004, Mini-Review in Organic Chemistry 1:93-102). The aforementioned enzymatic resolution of unprotected β -lactams, although providing products of high enantiomeric excess, suffers from poor yields and necessitate use of a full equivalent of water. The methods using N-acyloxymethyl ester protected lactams suffer from poor yields and low enantiomeric excess.

[0033] N-Benzoyl protected β -lactams have been enzymatically resolved using various enzymes, but not *Candida antarctica* (see Brieva et al., 1993, J. Org. Chem. 58(5):1068-1075). The benzoyl protected β -lactams were found to be unstable in aqueous media, undergoing hydrolysis without enzyme participation, presumably due to over-activation by the benzoyl group. Even so, when using organic media for the resolutions, good enantiomeric excess was achieved, however with poor yields.

[0034] In contrast, the inventors of the present application have discovered that the use of β -lactams in which the amide ring nitrogen atom is protected as a carbamate enhance reactions with lipases from *Candida antarctica* and provide stereoisomerically pure products in high yields. While not intending to be bound by any theory of operation, it is believed that the carbamate activates the β -lactam toward lipase catalysis, but not to the point of instability in aqueous media. It is believed that carbamate equivalents as described herein will also activate the β -lactam toward lipase catalysis without aqueous instability. This combination of enhanced reactivity and relative stability in aqueous media of the N-protected β -lactam, along with lipases from *Candida antarctica* provide novel means for producing stereoisomerically pure products in high yields.

[0035] Lipases from *Candida antarctica* are commercially available in variety of different forms and preparations, including resin-immobilized, lyophilized and suspended preparations, from, for example BioCatalytics (Pasadena, CA, USA), Novozyme (Franklinton, NC, USA), Sigma (St. Louis, MO, USA) and Aldrich Chemical Co.

(Milwaukee, WI, USA). As a specific example, suitable lipases from *Candida antarctica* are sold under the tradename Chirazyme by Roche Diagnostics Corp. (Indianapolis, IN). Although lyophilized and suspended preparations are useful in the methods described herein, the use of immobilized enzymes provides several advantages, including, but not limited to, improved enzyme stability, convenience of handling and ease with which the enzyme can be recycled. As a non-limiting example, Table 1 lists some of the lipases from *Candida antarctica* that are commercially available through BioCatalytics (Pasadena, CA, USA) that are suitable for use in the methods described herein.

Table 1	
Catalog No.	Product Description
IMB-101	Lipase from <i>Candida antarctica</i> , type B, carrier-fixed 1 (equivalent to Chirazyme L2, c-f)
IMB-102	Lipase from <i>Candida antarctica</i> , type B, carrier-fixed 2 (equivalent to Chirazyme L2, C2)
IMB-103	Lipase from <i>Candida antarctica</i> , type B, carrier-fixed 3 (equivalent to Chirazyme L2, C3)
IMB-104	Lipase from <i>Candida antarctica</i> , type A, carrier-fixed 1 (equivalent to Chirazyme L5, c-f)

[0036] Generally, each lipase enzyme exhibits some degree of unique thermostability, substrate specificity and/or chemospecificity. In some embodiments, lipases from *Candida antarctica* type B are used.

[0037] In some embodiments, a resin-immobilized lipase from *Candida antarctica* is used. When protected with a Boc group at the lactam amide nitrogen, it has been observed that the enzyme selectively binds and hydrolyzes the enantiomer of structural formula 2 with high specificity, but does not react with the enantiomer of structural formula 1. While not intending to be bound by any theory of operation, it is believed that the carbamate group activates the enzymatic resolution process. Moreover, the carbamate group, by virtue of its relative lipophilicity, aids in the separation of the resultant diastereomeric products, providing stereoisomerically pure products in high yields. It is expected that protecting groups other than Boc that have similar properties, and groups that are equivalent to carbamates as described herein, will yield similar results.

[0038] Suitable carbamates include, but are not limited to, those described above, including Boc, benzyloxycarbonyl and 9-fluorenylmethoxycarbonyl. Other useful carbamates can be found in Greene & Wuts., *Protective Groups in Organic Synthesis*, 3rd Ed., John Wiley & Sons, Inc., New York (1999) and the references cited therein (see, e.g., the myriad carbamates at pp. 503-550, the disclosure of which is incorporated herein by reference.) Any carbamate protecting groups in a β -lactam that can be cleaved without racemization of the chiral stereocenters in a stereoisomerically pure compound are useful.

[0039] In general, the method for generating stereoisomerically pure compounds comprises contacting an N-protected β -lactam comprising a mixture of enantiomers according to structural formulae 1 and 2 (for example, a racemic mixture) with a lipase enzyme from *Candida antarctica* in a reaction solvent. As mentioned above, the enzyme hydrolyzes one stereoisomer, but leaves the other stereoisomer intact. The amount of enzyme used is not critical and can be varied. Usually, a catalytic amount of enzyme is sufficient. In other situations, at least one molecule of enzyme per 500 molecules of substrate is desirous to achieve adequate resolution of N-protected β -lactams.

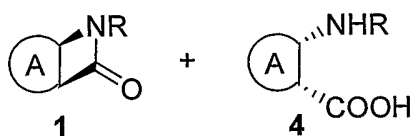
[0040] Similarly, the choice of reaction solvent can be varied, depending, in part, on the solubility of the starting materials and products. Examples of common solvents useful for the methods described herein include diisopropyl ether, tetrahydrofuran, butanol, hexanes, toluene and acetonitrile. Mixtures of these solvents may also be used. The use of organic solvents for enzymatic resolutions, rather than aqueous systems, is particularly useful. Since some water may be necessary to adequately hydrate the enzyme, a catalytic amount of water, for example, in the range of about 0.1-1.0% is advantageous. The catalytic amount of water can usually be achieved by using fresh commercially available anhydrous solvents without further distillation, as these solvents typically are not 100% water free. For a discussion of the importance and use of water in enzymatic reactions carried out in organic solvents, see Klibanov, 1997, "Why Are Enzymes Less Active In Organic Solvents Than In Water?" *Trends in Biotechnology* 15(3): 97-101, the disclosure of which is incorporated herein by reference.

[0041] Temperature also governs the reactivity and stereoselectivity of the enzyme. Generally, enzymes are susceptible to temperature. For practical applications, the stereoselective methods described herein can be carried out at a temperature in the range

of about 0-80 °C. In some embodiments it may be advantageous to use a temperature in the range of about 20-60 °C. The optimum temperature for carrying out any particular stereoselective reaction described herein can be determined by the skilled artisan by, for example, monitoring the progress of the reaction using analytical techniques such as, but not limited to, nuclear magnetic resonance, mass spectrometry, IR spectroscopy, UV/VIS absorption, optical polarimetry, GC chromatography, HPLC, or combinations thereof, over time.

5.3 Uses for Stereoisomerically Enriched β -Lactams

[0042] As mentioned above, lipases from *Candida antarctica* selectively hydrolyze the enantiomer of structural formula **2**, leaving the enantiomer of structural formula **1** intact. Hence, the product of the reaction is a mixture of compounds according to structural formulae **1** and **4**:

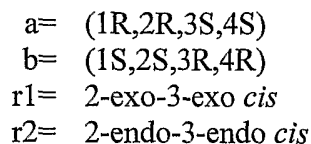


[0043] wherein A and R are as previously defined.

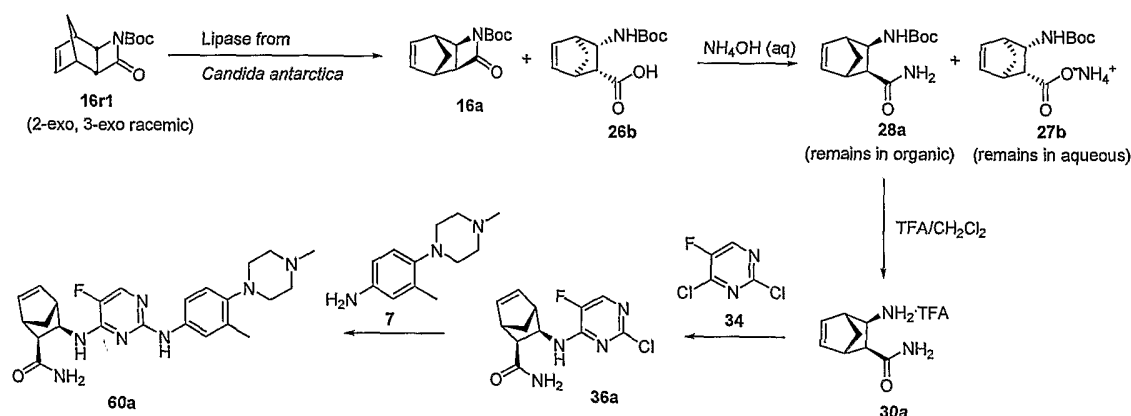
[0044] If desired, β -lactam **1** and N-protected β -amino carboxylic acid **4** can be isolated and/or purified using standard analytic techniques known to the skilled artisan, such as, but not limited to, electrophoresis, selective precipitation, fractional crystallization, ion exchange chromatography, high pressure liquid chromatography (HPLC), or distillation. In some embodiments, β -lactam **1** can be isolated from amino carboxylic acid **4** by converting one product to a water soluble form, for example a carboxylate salt, and separating the products based on their respective solubilities in aqueous and organic solvents. Specific examples of such separations are provided in the Examples sections.

[0045] Stereoisomerically pure β -lactam **1** and N-protected β -amino carboxylic acid **4** are both useful as starting materials to synthesize specific diastereomers of a wide variety of molecules. For example, stereoisomerically pure β -lactam **1** can be used as a starting material to synthesize diastereomerically pure antiproliferative (1R, 2R, 3S, 4S)-N4-(3-aminocarbonylbicyclo[2.2.1] hept-5-en-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine. An exemplary embodiment of the synthesis of this compound is illustrated in Scheme (I). In the various exemplary reaction

schemes discussed herein illustrated with 3-aminocarbonylbicyclo[2.2.1] hept-5-en-2-yl-2,4-pyrimidinediamine compounds, including Scheme (I), compound numbers followed by a suffix, such as **a**, **b**, **r1** and **r2**, refer to specific diastereomers and racemates, as follows:



Scheme (I)



[0046] Referring to Scheme (I), the enzymatic resolution of a racemic mixture of (2-exo, 3-exo) *cis* N-Boc protected β -lactam **16r1**, which comprises stereoisomers **16a** and **16b** (not illustrated) using a lipase from *Candida antarctica* yields β -lactam **16a** and N-Boc β -amino carboxylic acid derivative **26b**. Treatment of β -lactam **16a** with aqueous ammonium hydroxide gives N-Boc-amino carboxamide derivative **28a**, which is soluble in organic solution, and salt **27b**, which is soluble in aqueous solution. N-Boc-amino carboxamide derivative **28a** can be partitioned into organic solution. Deprotection of the Boc group with TFA yields amino carboxamide **30a**, which can now undergo a nucleophilic aromatic substitution reaction with 5-fluoro-2,4-dichloro-pyrimidine **34** to yield compound **36a**. Nucleophilic aromatic substitution of compound **36a** with aniline **7** yields diastereomerically pure (1R, 2R, 3S, 4S)-N4-(3-aminocarbonyl-bicyclo[2.2.1]hept-

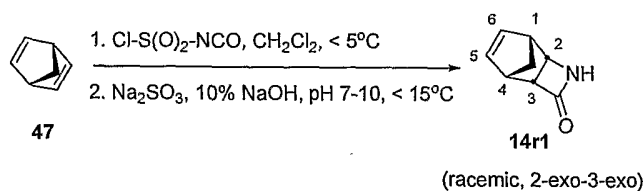
5-en-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine **60a**. Those of skill in the art will appreciate that the stereoisomeric configuration and optical purity of stereoisomer **16a** will, in most circumstances, determine the stereoisomeric configuration and optical purity of (1R, 2R, 3S, 4S)-N4-(3-aminocarbonyl-bicyclo[2.2.1]hept-5-en-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine **60a**.

[0047] Additional 3-substituted-cycloalkyl-2,4-pyrimidinediamine compounds that can be synthesized utilizing stereoisomerically pure β -lactam **1** as a starting material are described in copending application Serial No. 11/133,419 filed May 18, 2005, international application no. PCT/US05/17470 and application Serial No. _____, entitled "Stereoisomerically Enriched 3-Aminocarbonyl Bicycloheptene Pyrimidinediamine Compounds and Their Uses," filed concurrently herewith (identified by attorney docket no. 375462-039US), the disclosures of which are incorporated herein by reference.

6. EXAMPLES

[0048] The inventions described herein are further defined by reference to the following examples, which describe the preparation of various compounds described herein, methods for assaying their biological activity, and methods for their use. It will be apparent to one of ordinary skill in the art that many modifications, both to the materials and methods, may be practiced without departing from the scope of the invention.

6.1 Preparation of 3-Aza-4-oxo-tricyclo[4.2.1.0(2,5)]non-7-ene

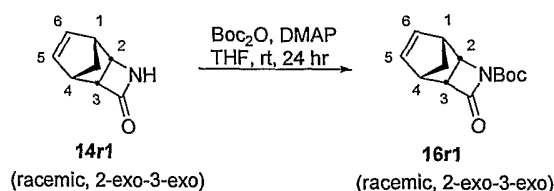


[0049] **Procedure: Part 1:** A solution of 2,5-norbornadiene **47** (25.0 mL, 0.246 mole) in CH_2Cl_2 (110 mL, fresh bottle) was cooled in an ice/ NaCl bath (-10°C). To this was added drop-wise a solution of CSI (21.4 mL, 0.246 mole) in CH_2Cl_2 (45 mL, fresh bottle) at a rate to maintain the temperature below 5°C (the addition took approx. 1.25 hr.). Upon completion of the addition, the reaction mixture was stirred for 1 hour at $0-5^\circ\text{C}$ and then removed from the cooling bath and allowed to warm to 20°C . The reaction mixture

was quenched with water (60 mL) and vigorously stirred for several minutes. The organic layer was separated, washed with brine, and dried with Na_2SO_4 . Concentration gave a light brown oil.

[0050] Part 2: A mixture of Na_2SO_3 (24.5 g), water (70 mL), and CH_2Cl_2 (30 mL) was cooled in an ice/ NaCl bath. The oil from **Part 1** was diluted to 100 mL with CH_2Cl_2 and added dropwise to the above mixture at a rate to maintain the temperature below 15°C (the addition took approx. 1.75 hr). The pH of the reaction mixture was monitored with a pH meter and kept basic (pH 7-10) by adjusting with 10% NaOH (w/v) (as needed). Upon completion of the addition, the reaction mixture was stirred for 1 hour at $5-10^\circ\text{C}$ (final pH was 8.5). The reaction mixture was poured into a separatory funnel and the CH_2Cl_2 layer separated. This organic phase was a thick and gelatinous solid suspension. It was diluted with water (approx. 400 mL) to make a more free flowing solution. The aqueous layer was further extracted with CH_2Cl_2 (4 x 100 mL). (Alternatively, the solids can be separated from the CH_2Cl_2 by centrifugation. The solids can then be diluted with water (until almost all dissolved) and extracted with CH_2Cl_2). The aqueous layer was further extracted with CH_2Cl_2 (10 X 100 mL). The CH_2Cl_2 extracts were monitored by TLC for the presence of product. The combined organic extracts were washed with brine, dried with MgSO_4 , and filtered through diatomaceous earth (celite). Removal of solvent gave the desired product, racemic-2-exo-3-endo 3-aza-4-oxo-tricyclo[4.2.1.0(2,5)]non-7-ene **14r1** as white solid (20.5 g, 62%). ^1H NMR ($\text{DMSO}-d_6$): δ 8.01 (bs, 1H), 6.22 (dd, $J=3.3$ and 5.4 Hz, 1H), 6.12 (dd, $J=3.3$ and 5.4 Hz, 1H), 2.88 (dd, $J=1.5$ and 3.3, 1H), 2.79 (bs, 1H), 2.74 (bs, 1H), 1.58 (d, $J=9.3$ Hz, 1H), and 1.47 (d, $J=9.3$ Hz, 1H).

6.2 Preparation of 4-Oxo-3-tert-butoxycarbonylaza-tricyclo[4.2.1.0(2,5)]non-7-ene

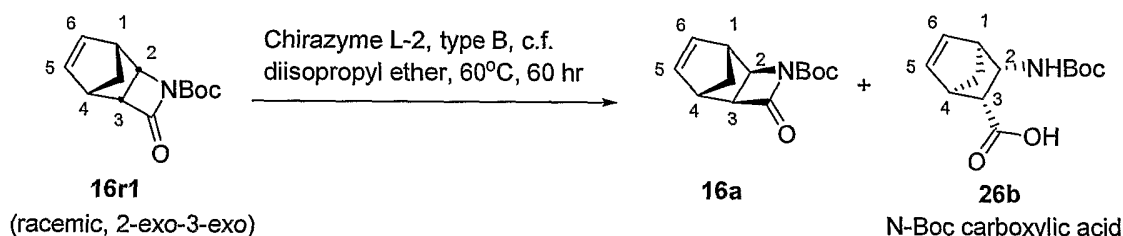


[0051] Procedure: A homogeneous mixture of 3-aza-4-oxo-tricyclo[4.2.1.0(2,5)]non-7-ene (**14r1**; racemic-2-exo-3-exo; 10.0 g, 74 mmol), $(\text{Boc})_2\text{O}$ (16.1 g, 74 mmol) and DMAP (1.1 g) in CH_2Cl_2 was stirred under N_2 at room temperature for 24 hours. To this reaction mixture were added EtOAc (100 mL) followed by H_2O (100 mL) and stirred for

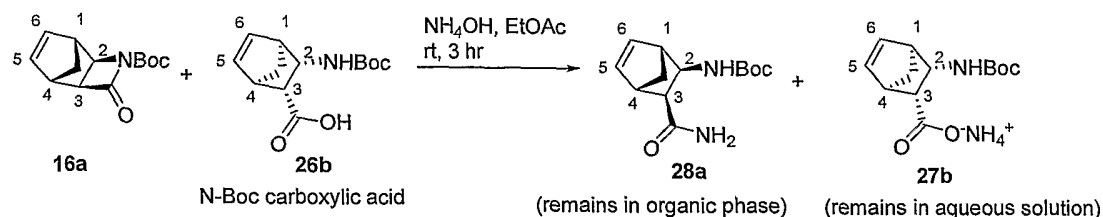
additional 1 hour. The organic layer was separated and washed with H₂O (2 x 100 mL). The organic layer was dried over anhydrous Na₂SO₄ and solvent was removed under a reduced pressure to afford 4-oxo-3-tert-butoxycarbonylaza-tricyclo[4.2.1.0(2,5)]non-7-ene (**16r1**; racemic-2-exo-3-exo) (16.5 g, 70%); ¹H NMR (DMSO-*d*₆): δ 6.29 (dd, J= 3.3 and 5.4 Hz, 1H), 6.19 (dd, J= 3.3 and 5.4 Hz, 1H), 3.77 (d, J= 4.5 Hz, 1H), 3.13 (bs, 1H), 3.08-3.04 (m, 1H), 2.93 (bs, 1H), 1.45 (s, 9H). LCMS: 95%

6.3 Enzymatic Preparation of Stereoisomerically Pure (1R, 2R, 3S, 4S)-N4-(3-Aminocarbonylbicyclo[2.2.1]hept-5-en-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine Using Chirazyme

6.3.1 Preparation of Stereochemically Pure N-Boc-β-Lactam



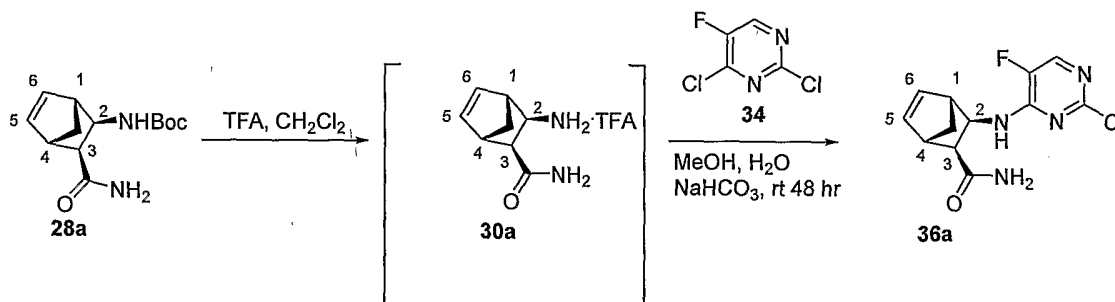
[0052] **Procedure:** A dry sealed tube charged with 4-oxo-3-tert-butoxycarbonylaza-tricyclo[4.2.1.0(2,5)]non-7-ene (**16r1**; racemic-2-exo-3-exo) (4.0 g, 17.02 mmol), resin bound/immobilized chirazyme L-2, type B, c.f. (8.0 g, purchased from BioCatalytics Inc., Pasadena, CA) and diisopropyl ether (80 mL) was gently shaken in an incubator at 60 °C for 60 hours. (The enzymatic resolution of racemic N-Boc β-lactam **16r1** was followed by proton NMR. The integration of tert-butyl group of enantiomerically pure N-Boc lactam **16a** and N-Boc carboxylic acid **26b** was seen in 1:1 ratio). The resulting reaction mixture was filtered and the solid resin was washed with diisopropyl ether (2 x 40 mL). The filtrate was concentrated to afford a mixture of enantiomerically pure N-Boc-β-lactam **16a** and N-Boc carboxylic acid **26b** (total mass: 4.0 gm).



[0053] **Procedure:** A round bottom equipped with a rubber septum and a magnetic stirring bar was charged with a mixture of enantiomerically pure N-Boc-lactam **16a** and

N-Boc carboxylic acid **26b** (4.0 g) under a positive pressure of nitrogen. To this were added ethyl acetate (50 mL) followed by 25% aqueous ammonium hydroxide (50 mL) and stirred at room temperature for 3 hours. The reaction progress was monitored by TLC. The ethyl acetate layer was separated and washed with 5% aqueous solution of NaHCO_3 (40 mL), dried over anhydrous Na_2SO_4 and solvent was evaporated to afford 2.00 gm (7.93 mmol out of a theoretical 8.51 mmol; 93% yield) of the desired enantiomerically pure N-Boc carboxamide **28a** with greater than 99% enantiomeric excess, as determined by chiral HPLC. The aqueous solution containing the N-Boc ammonium carboxylate **27b** upon acidification with cold 1N HCl followed by extraction with CH_2Cl_2 regenerated the N-Boc carboxylic acid **26b** (1.8 g, 7.11 mmol out of a theoretical 8.51 mmol, 84% yield). ^1H NMR (DMSO-d_6): 7.26 (bs, 1H), 6.66 (bs, 1H), 6.13 (m, 2H), 3.59 (t, 1H, $J = 6.9$ Hz), 2.80 (s, 1H), 2.54 (s, 1H), 2.31 (d, 1H, $J = 8.1$ Hz), 2.00 (d, 1H, $J = 8.7$ Hz), 1.36 (s, 9H), 1.30 (d, 1H, $J = 8.1$ Hz); LCMS: MS (m/z): 254 (MH^+); $[\alpha]_D -76.78^\circ$ (c 1.0, MeOH).

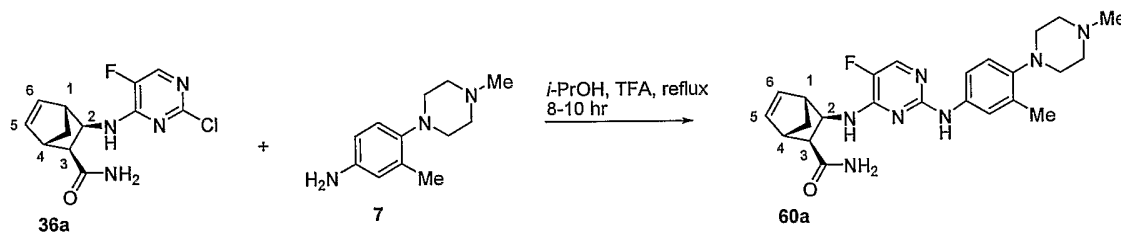
6.3.2 Preparation of Stereoisomerically Pure Mono SNAr Product



[0054] **Procedure:** A round bottom flask equipped with N_2 inlet and a magnetic stirring bar was charged with enantiomerically pure N-BOC carboxyamide **28a** (2.00 g, 7.93 mmol) and then treated with 20% of TFA in CH_2Cl_2 at room temperature for 2 hours. The reaction progress was monitored by TLC. The resulting solution was concentrated under a reduced pressure. The trace of TFA was removed under high vacuum for several hours to afford the enantiomerically pure intermediate, TFA salt **30a** in quantitative yield. ^1H NMR (DMSO-d_6): 8.10 (bs, 2H), 7.92 (s, 1H), 7.25 (s, 1H), 6.29 (m, 1H), 6.18 (m, 1H), 4.38 (bs, 1H), 3.06 (d, 1H, $J = 7.2$ Hz), 2.97 (s, 1H), 2.87 (s, 1H), 2.43 (d, 1H, $J = 7.5$ Hz), 2.10 (d, 1H, $J = 6$ Hz), 1.36 (d, 1H, $J = 8.7$ Hz); LCMS: MS (m/z): 152 (MH^+).

[0055] The resulting TFA salt **30a** was treated with 2,4-dichloro-5-fluoropyrimidine **34** (1.58 g, 9.51 mmol) in MeOH:H₂O (20:10 mL) in the presence of NaHCO₃ (1.33 g, 15.84 mmol) at room temperature for 48 hours. The reaction mixture was diluted with H₂O (25 mL), saturated with NaCl and extracted with EtOAc (3 x 50 mL). Upon drying over anhydrous Na₂SO₄ the solvent was evaporated and the residue was chromatographed (silica gel, CH₂Cl₂ then 2-4% 2N NH₃/MeOH in CH₂Cl₂) to afford 2.02 g (91%) of desired mono-SNAr product **36a**. ¹H NMR (DMSO-d₆): 8.25 (d, 1H, J= 7.2 Hz), 8.07 (d, 1H, J=3.3 Hz), 7.71 (s, 1H), 7.19 (s, 1H), 6.29 (m, 2H), 3.99 (t, 1H, J= 7.8 Hz), 2.85 (s, 1H), 2.75 (s, 1H), 2.49 (d, 1H, J= 0.9 Hz), 2.11 (d, 1H, J= 8.7 Hz), 1.39 (d, 1H, J= 8.7 Hz); LCMS: purity: 95%, MS (m/z): 283 (MH⁺). The enantiomeric purity was greater than 99% as determined by chiral HPLC; [α]_D + 61.10° (c 1.0, MeOH).

6.3.3 Preparation of Stereoisomerically Pure (1R,2R,3S,4S)-N4-(3-Aminocarbonylbicyclo[2.2.1]hept-5-en-2-yl)-5-fluoro-N2-[3-methyl-4-(4-methylpiperazin-1-yl)phenyl]-2,4-pyrimidinediamine



[0056] **Procedure:** A dry reaction flask equipped with a stirring bar, reflux condenser and an N₂ inlet was charged with enantiomerically pure mono-SNAr product **36a** (2.25 g, 8 mmol), aniline **7** (1.80 g, 8.8 mmol), TFA (1.12 mL) and isopropanol (18 mL) and the resulting reaction mixture was stirred at reflux temperature for 8-10 hours. After cooling the reaction mixture to room temperature, ethyl acetate (20 mL) was added. The solid obtained was filtered and washed with ethyl acetate (2 x 5 mL) to afford compound **60a** in the form of acidic salt. The resulting solid was then taken into water and the aqueous mixture adjusted to pH 9 with aqueous NaHCO₃, which caused precipitation of a solid. The solid was filtered from the mixture, washed with water and dried to give 3.3 g (93%) of 2,4-pyrimidinediamine derivative **60a**. ¹H NMR (DMSO-d₆): 8.85 (s, 1H), 7.83 (d, 1H, J= 2.7 Hz), 7.68 (s, 1H), 7.47 (s, 2H), 7.36 (d, 1H, J= 7.8 Hz), 7.18 (s, 1H), 6.89 (d, 1H, J= 8.7 Hz), 6.32 (m, 1H), 6.25 (m, 1H), 4.11 (t, 1H, J= 7.8 Hz), 3.32 (s, 3H), 2.86 (s, 1H), 2.76 (m, 4H), 2.49 (m, 4H), 2.46 (m, 2H), 2.21 (s, 3H), 2.11 (d, 1H, J= 8.4 Hz), 1.39

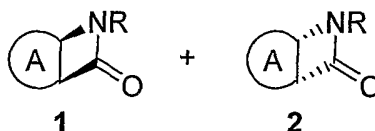
(d, 1H, J= 9Hz); LCMS: purity: 100 %, MS (m/z): 452 (M^+); > 99 %ee as determined by chiral HPLC; $[\alpha]_D^{RT} +101.2^\circ$ (c 1.0, MeOH).

[0057] Although the foregoing inventions have been described in some detail to facilitate understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims. Accordingly, the described embodiments are to be considered as illustrative and not restrictive, and the invention is not to be limited to the details given herein, but may be modified within the scope and equivalents of the appended claims.

[0058] All literature and patent references cited throughout the application are incorporated into the application by reference for all purposes.

WHAT IS CLAIMED IS:

1. A method for generating a stereoisomerically pure N-protected β -lactam from a mixture of diastereomers, comprising contacting an N-protected β -lactam mixture comprising enantiomers according to structural formulae (1) and (2):

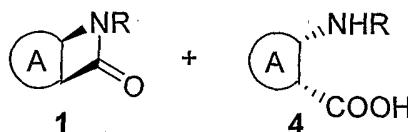


wherein:

A represents a saturated or unsaturated, monocyclic, polycyclic or bridged polycyclic ring; and

R is a carbamate or thiocarbamate protecting group,

with a lipase from *Candida antarctica* under conditions in which the lipase selectively cleaves enantiomer **2**, thereby yielding a mixture of reaction products according to structural formulae **1** and **4**:



2. The method of **claim 1**, wherein the carbamate protecting group is of the formula $-\text{C}(\text{O})\text{OR}^1$, where R^1 is selected from unsubstituted or substituted alkyl, unsubstituted or substituted (C6-C20) aryl and substituted or unsubstituted (C7-C26) arylalkyl.

3. The method of **claim 2**, wherein R^1 is selected from *t*-butyl and benzyl.

4. The method of **claim 1**, wherein **A** is selected from bicycloheptenyl, bicycloheptyl, cycloheptyl, cycloheptenyl, cyclohexyl, cyclohexenyl, cyclopentyl, cyclopentenyl, cyclopropyl, and cyclobutyl.

5. The method of **claim 1**, wherein the lipase is a type B lipase.

6. The method of **claim 1**, wherein the lipase is bound to a resin.

7. The method of **claim 1**, wherein a catalytic amount of the lipase is used.

8. The method of **claim 1**, wherein the contacting is carried out at a temperature in the range of about 0-80 °C.

9. The method of **claim 8**, wherein the temperature in the range of about 20-60 °C.

10. The method of **claim 1**, wherein the contacting is carried out in a solvent selected from diisopropyl ether, tetrahydrofuran, butanol, toluene, hexanes, acetonitrile, and mixtures thereof.

11. The method of **claim 11**, wherein the solvent has a water content in the range about of 0.1 to 1.0 wt%.

12. The method of **claim 1**, which further comprises the step of isolating compound **1** from compound **4**.

13. The method of **claim 12**, wherein the isolation is carried out by acid-base extraction using aqueous and organic solvents.