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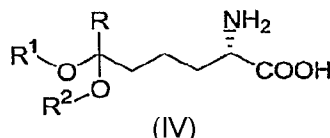
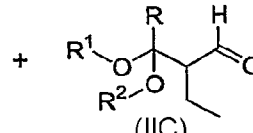
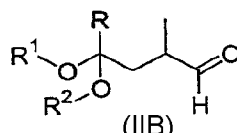
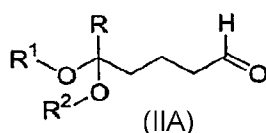
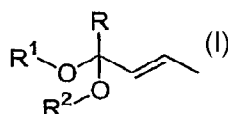
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(54) Title: PROCESS FOR MAKING AMINO ACIDS



(57) **Abstract:** A process for making an amino acid by the steps of: (a) contacting a compound of formula I with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc; (b) reacting the mixture of aldehyde compounds from step (a) to produce a mixture of derivative compounds comprising a masked amino acid derivative which may be selected from the group consisting of amino nitrile, N-acyl amino acid, amino amide, hydantoin and amino ester; (c) contacting the mixture of derivative compounds from step (b) with an enantioselective hydrolase enzyme in the presence of water to produce an L-amino acid having the formula IV; (d) isolating the amino acid having the formula IV in substantially pure form, wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc and IV, R is H, alkyl or aryl and R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused.

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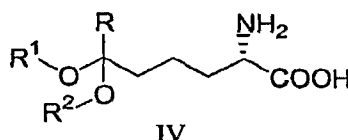
PROCESS FOR MAKING AMINO ACIDS

FIELD OF THE INVENTION

This invention is in the field of methods for the synthesis of amino acids.

BACKGROUND OF THE INVENTION

Certain L-amino acids having are suited for use as intermediates in the pharmaceutical industry (see for example US6329542 and WO02/042258). These L-amino acids, such as those having the formula IV,



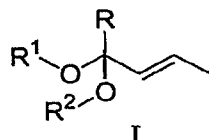
wherein R is H, alkyl or aryl and R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused, can be synthesized by using mono-acetal—aldehydes or ketal aldehydes. However, synthesis of mono-acetal-aldehydes from dialdehydes suffers from complications in purification and low yields, particularly when the two formyl groups are equivalent. It is even more difficult to make ketal-aldehydes from ketoaldehydes because the formyl group is more reactive than the keto group. Hydroformylation of readily available olefinic acetals or olefinic ketals typically produces a desired linear aldehyde and undesired branched aldehydes. Separation of the linear mono-acetal aldehyde and the branched mono-acetal-aldehydes is generally very difficult due to high, similar boiling points.

SUMMARY OF THE INVENTION

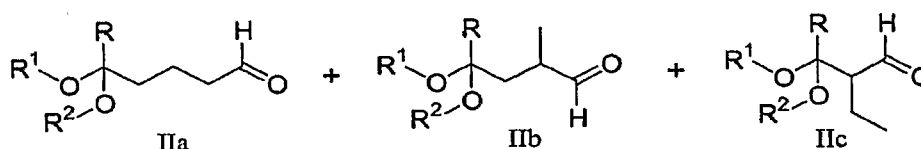
We have discovered that it is not necessary to separate the branched aldehydes from the linear aldehyde if, for example, a subsequent formation of a masked amino acid derivative and catalytic bio-resolution steps are used to convert the linear aldehyde selectively to the desired final product. The masked amino acid derivative may be selected from the group consisting of amino nitrile, N-acyl amino acid, amino amide, hydantoin and amino ester. The catalytic bio-resolution may be effected by exposure of said derivative to an enantioselective hydrolase enzyme selected from the group consisting of

nitrilases, nitrilase hydratases, aminoacylases, amidases, hydantionases, esterases and other hydrolase enzymes having equivalent activity to the aforementioned.

In general, the instant invention is process for making an amino acid, comprising the steps of: (a) contacting a compound of formula I

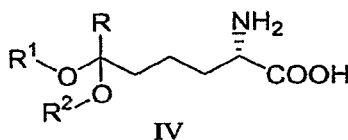


with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc;



(b) reacting the mixture of aldehyde compounds from step (a) to produce a mixture of derivative compounds comprising a masked amino acid derivative which may be selected from the group consisting of amino nitrile, N-acyl amino acid, amino amide, hydantoin and amino ester.;

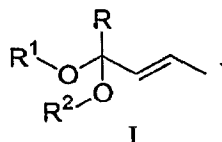
(c) contacting the mixture of derivative compounds from step (b) with an enantioselective hydrolase enzyme in the presence of water to produce an L-amino acid having the formula IV;



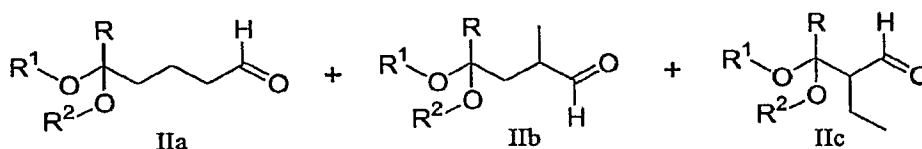
(d) isolating the amino acid having the formula IV in substantially pure form, wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc and IV, R is H, alkyl, alkylaryl, aryl or arylalkyl and R¹ and R² are the same or different alkyl groups preferably of up to 6 carbon atoms and more preferably methyl or ethyl and wherein R¹ and R² may be fused (i.e. R¹ and R² taken together may form a cyclic group with the O-C-O group to which they are linked), preferably to form a 1,3-dioxolane or 1,3-dioxane group and more preferably to form a 1,3-dioxolane group. Preferably, if R is an alkyl it has up to 12 carbon atoms, more preferably up to 8 carbon atoms most preferably up to 6 carbon atoms; if R is arylalkyl it has up to 20 carbon atoms, preferably up to 12 carbon atoms; if R is an aryl it has up to 16 carbon atoms,

more preferably up to 10 carbon atoms; and if R is alkylaryl it has up to 20 carbon atoms, preferably up to 12 carbon atoms.

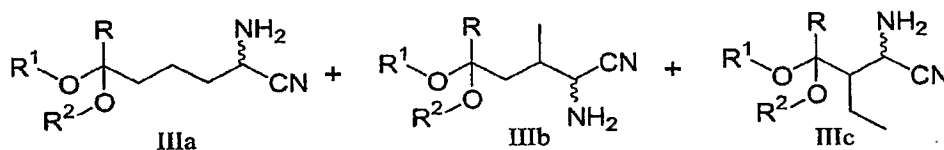
In one embodiment, the instant invention is a process for making an amino acid, comprising the steps of: (a) contacting a compound of formula I



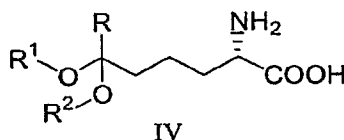
with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc;



(b) contacting the mixture of aldehyde compounds with a Strecker reagent to produce a mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc; and



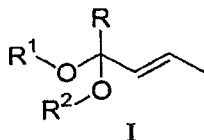
(c) contacting the mixture of nitrile compounds with an L-specific nitrilase in the presence of water to produce an L-amino acid having the formula IV, and



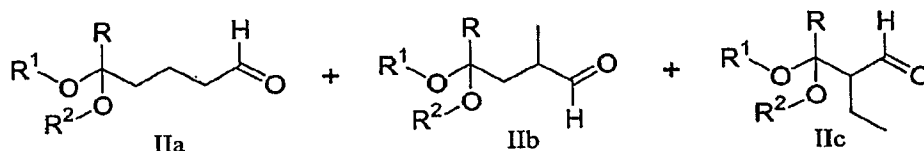
(d) isolating, preferably by precipitation, the L-amino acid having the formula IV, in substantially pure form,

wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc and IV, R, R¹, and R² are as defined above.

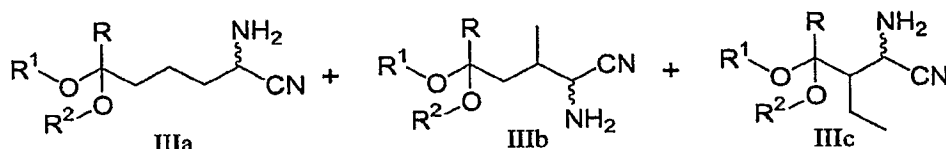
In another embodiment, the instant invention is a process for making an amino acid, comprising the steps of: (a) contacting a compound of formula I



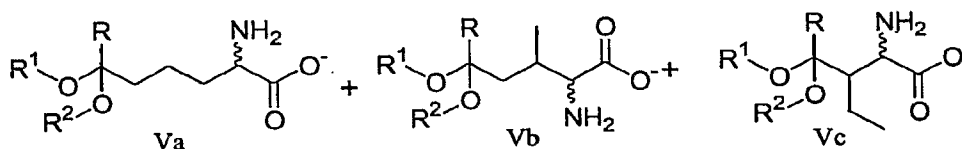
with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc;



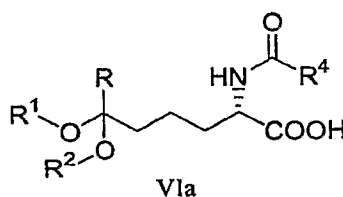
(b) contacting the mixture of aldehyde compounds with a Strecker reagent to produce a mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc;



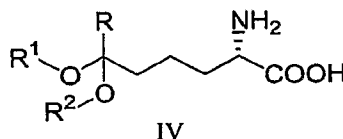
further contacting the mixture of nitrile compounds with aqueous ethanol under basic conditions to produce a mixture of amino acid salts comprising the formulas Va, Vb and Vc;



further contacting the mixture of amino acid salts with an acylating reagent to produce an acetal compound comprising the formula VIa; and



(c) contacting the acetal compound with an L-specific aminoacylase in the presence of water to produce an L-amino acid having the formula IV, and

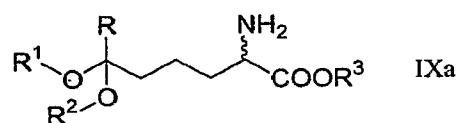
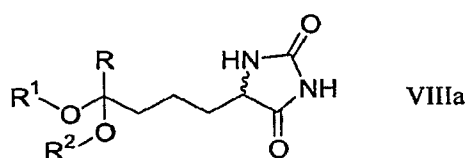
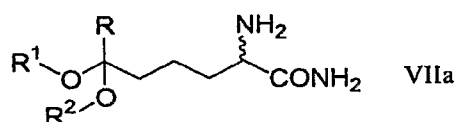


(d) isolating, preferably by precipitation, the L-amino acid having the formula IV, in substantially pure form,

wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc, Va, Vb, Vc, VIa and IV, R, R¹, and R² are as defined above and , wherein R⁴ is an an alkyl, aryl or alkylaryl group containing from one to ten carbons.

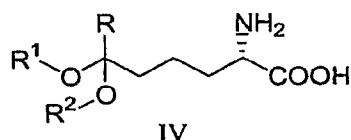
In another embodiment of the instant invention, the mixture of aldehyde compounds IIa, IIb and IIc can be further reacted such that linear aldehyde IIa is converted to a masked amino acid derivative selected from an amino amide having the formula VIIa, a hydantoin

having the formula VIIIa or an amino ester having the formula IXa, wherein R^3 is an alkyl or aralkyl group containing from one to ten carbons and wherein R^1 and R^2 are as defined above. Such derivative is then subject to enantioselective catalytic bio-resolution to produce the L-amino acid of formula IV which is then isolated, preferably by precipitation, to produce purified L-amino acid of formula IV.



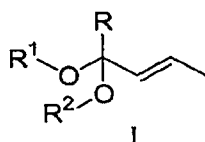
DETAILED DESCRIPTION

The instant invention in one embodiment is a process for making an L-amino acid having the formula IV, wherein R, R^1 and R^2 are as defined above.

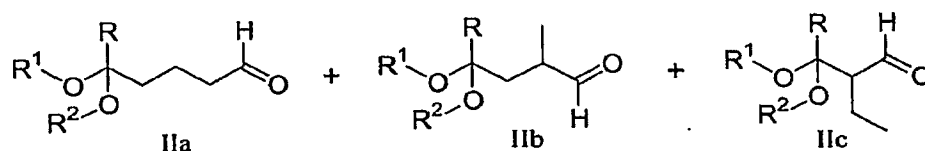


L-amino acids having the formula IV are suited for use as intermediates in the pharmaceutical industry.

The process of the instant invention comprises four steps. The first step is to contact a compound of formula I



with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc (and may contain small amounts of other by products, such as from hydrogenation and aldol condensation), wherein R, R¹ and R² are as defined above. Aldehyde IIa is formed via in situ isomerisation of compound I to the corresponding terminal olefin.



Hydroformylation of olefins to aldehydes using synthesis gas is well known, see, for example, Klein et al., *Angew. Chem. Int. Ed.* 2001, 40, No. 18, 3408-3411. In the instant invention, the synthesis gas is preferably comprises hydrogen and carbon monoxide in a mole ratio of of about 2:1 to 1:2. Although the specific hydroformylation catalyst used is not critical in the instant invention (see for example, the hydroformylation catalyst of Cuny et al., *J. Am. Chem. Soc.* 1993 2066-2068 or Selent et al., *Ang. Chem. Int. Ed.* 2001, 40, No. 9, 1696-1698), preferably, the hydroformylation catalyst is sufficiently selective to maximize the production of the linear aldehyde of formula IIa relative to the branched aldehydes of formulas IIb and IIc. More specifically, the hydroformylation catalyst selected for use in the instant invention preferably results in a mole ratio of compound of formula IIa to the compounds of formulas IIb and IIc of at least 3:1. The hydroformylation catalyst selected for use in the instant invention more preferably results in a mole ratio of compound of formula IIa to the compounds of formulas IIb and IIc of at least 6:1. The hydroformylation catalyst selected for use in the instant invention even more preferably results in a mole ratio of compound of formula IIa to the compounds of formulas IIb and IIc of at least 9:1.

WO 03/078444 A2 and US4668651 teach several highly preferred hydroformylation catalysts suitable for use in the instant invention specifically including a rhodium/Biphephos hydroformylation catalyst which will be described below (EXAMPLE 1) in greater detail.

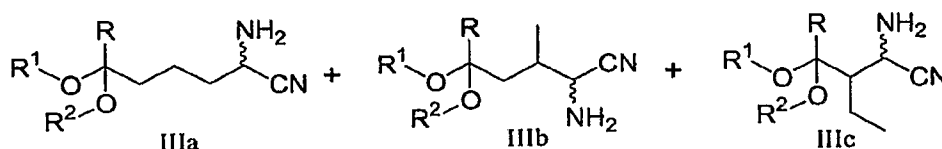
The hydroformylation reaction is preferably carried out in an aprotic polar solvent such as tetrahydrofuran. Then, a nonpolar solvent such as hexane is preferably added to the reaction mixture to form a mixture of the aldehyde compounds IIa, IIb and IIc in a mixture comprising the aprotic polar solvent and the nonpolar solvent. In this preferred embodiment, the mixture is then extracted with water to form an aqueous phase comprising the mixture of

aldehyde compounds IIa, IIb and IIc and the aprotic polar solvent and an organic phase comprising the hydroformylation catalyst and the nonpolar solvent so that the hydroformylation catalyst can be separated from the mixture of aldehyde compounds.

The specific aprotic polar solvent used for the hydroformylation reaction of the instant invention is not critical. However, preferably the aprotic polar solvent has an aqueous-hexane partition coefficient in the range of from 8:1 to 1:2, and more preferably in the range of from 4:1 to 1:1 (and most preferably about 2:1). The solvent can, preferably, be easily evaporated from the aqueous product mixture. The temperature of the hydroformylation reaction of the instant invention is preferably in the range of from 30 to 120 degrees Celsius, and more preferably in the range of from 40 to 100 degrees Celsius, and most preferably in the range of from 50 to 90 degrees Celsius. The synthesis gas used preferably has a carbon monoxide (CO) to hydrogen (H₂) mole ratio of 1:1. The pressure of the synthesis gas used in the hydroformylation reaction is preferably in the range of from 1 to 200 pounds per square inch gauge pressure (psig), and more preferably in the range of from 5 to 100 psig, and most preferably in the range of from 10-50 psig.

The aqueous solution of the aldehyde compounds comprising formulas IIa, IIb, and IIc can be used directly in the subsequent Strecker reaction as described hereinbelow. Alternatively, the aldehyde compounds IIa, IIb and IIc in the aqueous phase are then preferably extracted from the aqueous phase with a volatile solvent, for example, methylene chloride which is then dried with, for example, magnesium sulfate, followed by evaporation of the volatile solvent to yield a product which comprises the aldehyde compounds IIa, IIb and IIc. The mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc is preferably analyzed by gas chromatography/mass spectroscopy (GCMS).

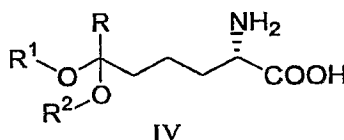
The mixture of aldehyde compounds is then contacted with a Strecker reagent to produce a mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc.



The specific Strecker reagent used is not critical in the instant invention. However, a Strecker reagent comprised of potassium cyanide or sodium cyanide, ammonium chloride, ammonium hydroxide and water is preferred and such a reagent will be described below in greater detail. Within this preferred embodiment, the Strecker reagent is normally combined

with the mixture of aldehyde compounds at about 0 degrees Celsius or lower and then the reaction mixture allowed to warm to about ambient temperature in order to complete the reaction. The nitrile compounds IIIa, IIIb and IIIc are then preferably extracted with a volatile solvent, such as ethyl acetate. This extract is then dried with a drying agent, such as magnesium sulfate, and then the volatile solvent is preferably evaporated to produce a product comprising the nitrile compounds IIIa, IIIb and IIIc. The mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc is preferably analyzed by gas chromatography/mass spectroscopy (GCMS).

According to one embodiment, the mixture of nitrile compounds IIIa, IIIb and IIIc is then contacted with an L-specific nitrilase to produce an L-amino acid having the formula IV, wherein R, R¹ and R² are as defined above. The L-amino acid having the formula IV is preferably assayed by chiral HPLC.

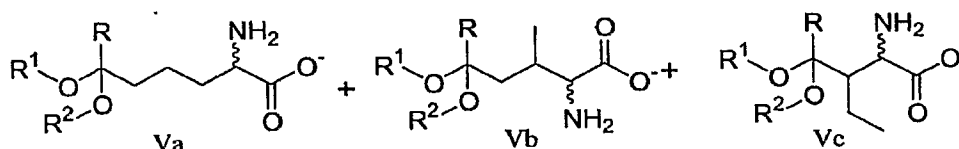


When 4-[1,3]dioxolan-2-yl-butyraldehyde is the compound of formula IIa, then L-allysine ethylene acetal is the compound of formula IV. This reaction preferably occurs at or near ambient temperatures (preferably about 10-40 degrees Celsius, more preferably about 15-30 degrees Celsius). The pH is controlled as appropriate for the enzyme and preferably is maintained at about pH 7-8. To achieve favorable process economics, substrate concentration is preferably at least 50 g/L and more preferably at least 100 g/L. To achieve such substrate concentrations, a water miscible co-solvent may be required, preferably methanol or alternatively ethanol, tetrahydrofuran, 1,2-dimethoxyethane or 1,4-dioxane.

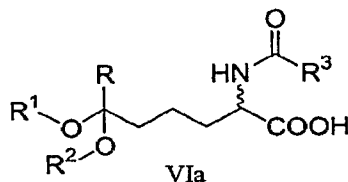
The specific L-specific nitrilase used is not critical in the instant invention. Nitrilases effective in the hydrolysis of the L-isomer of nitrile compound IIIa are described in US20040002147. An important benefit of the instant invention is the fact that L-specific nitrilase will not catalyze the hydrolysis of the nitrile compounds IIIb or IIIc or the D isomer of the nitrile compound IIIa. In addition, the solubility of the L-amino acid IV produced is generally relatively low in the buffers used for the nitrilase so that the L-amino acid IV conveniently precipitates from solution in a high purity crystalline form.

As an alternative embodiment of the instant invention, the mixture of nitrile compounds IIIa, IIIb and IIIc can be contacted with aqueous ethanol (or another water

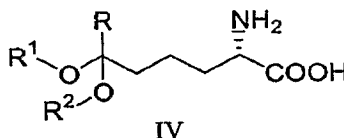
miscible solvent such as methanol, ethanol, tetrahydrofuran, 1,2-dimethoxyethane or 1,4-dioxane) under basic conditions to produce a mixture of amino acid salts comprising the formulas Va, Vb and Vc. Preferably, the reaction mixture is heated at or near reflux temperatures. The mixture of amino acid salts comprising the formulas Va, Vb and Vc is preferably analyzed by high performance liquid chromatography (HPLC).



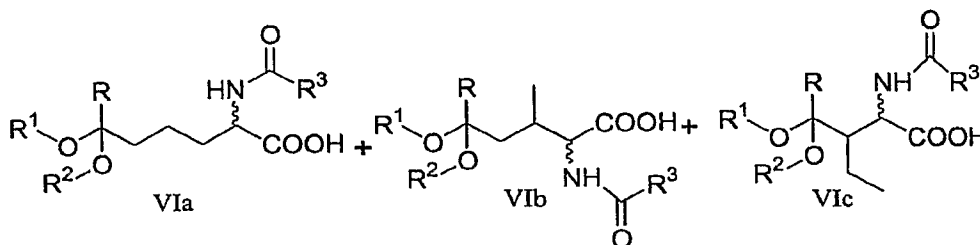
The mixture of amino acid salts can then be contacted with an acylating reagent, such as acetic anhydride or benzoyl chloride, to produce an acetal compound comprising the formula VIa. The acetal compound is then preferably extracted with a volatile solvent, such as ethyl acetate. This extract is then dried with a drying agent, such as magnesium sulfate, and then the volatile solvent is preferably evaporated to produce a product comprising the acetal compound VIa. The acetal compound comprising the formula VIa is preferably analyzed by high performance liquid chromatography (HPLC).



The acetal compound is then be contacted with an L-specific aminoacylase to produce the L-amino acid having the formula IV, wherein, again, R, R¹, and R² are as defined above.



When the mixture of amino acid salts is contacted with the acylating reagent a mixture of acetal compounds comprising the formulas VIa, VIb and VIc are produced.



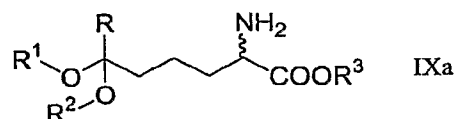
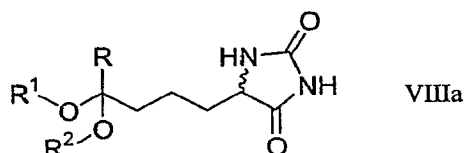
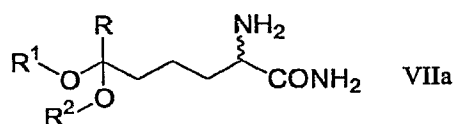
The mixture of acetal compounds are then preferably extracted with a volatile solvent, such as ethyl acetate. This extract is then dried with a drying agent, such as magnesium sulfate, and then the volatile solvent is preferably evaporated to produce a product comprising the acetal compounds VIa, VIb and VIc. The acetal compounds comprising the formulas VIa, VIb and VIc are preferably analyzed by high performance liquid chromatography (HPLC).

When the mixture of acetal compounds VIa, VIb and VIc forms a precipitate, then it is preferable to wash such precipitate with a solvent (such as methyl t-butyl ether) to dissolve compounds VIb and VIc from VIa, thereby leaving compound VIa in pure or purer form for contact with the L-specific aminoacylase.

The specific L-specific aminoacylase used is not critical in the instant invention. Preferably a thermophilic aminoacylase is used, allowing operating temperatures of about 40-70 degrees Celsius and thereby achieving faster reaction and/or higher substrate concentration of substrate. A conventional aminoacylase may also be employed at typical operating temperatures of about 15-45 degrees Celsius. For both thermophilic and conventional aminoacylases, stringent pH control during the reaction is not required although a starting pH in the range 6-9 is preferred. Also, substrate concentration is preferably at least 50 g/L and more preferably at least 100 g/L. An important benefit of the instant invention is the fact that L-specific aminoacylase will not catalyze the hydrolysis of any acetal compounds VIb or VIc or the D isomer of compound VIa. In addition, the solubility of the L-amino acid IV produced is generally relatively low in the buffers used for the acylase so that the L-amino acid IV conveniently precipitates from solution in a high purity crystalline form. The L-amino acid having the formula IV is preferably assayed by chiral HPLC.

As alternative embodiments of the instant invention, the mixture of aldehyde compounds IIa, IIb and IIc, obtained by hydroformylation, can be further reacted such that linear aldehyde IIa is converted to a masked amino acid derivative selected from an amino amide having the formula VIIa, a hydantoin having the formula VIIIa or an amino ester having the formula IXa. Conversion of linear aldehyde IIa to amino amide VIIa can for example be carried out by conversion to the amino nitrile IIIa as previously described, followed by partial hydrolysis of the nitrile group under basic conditions. Conversion of linear aldehyde IIa to hydantoin VIIIa can for example be carried out by reaction with

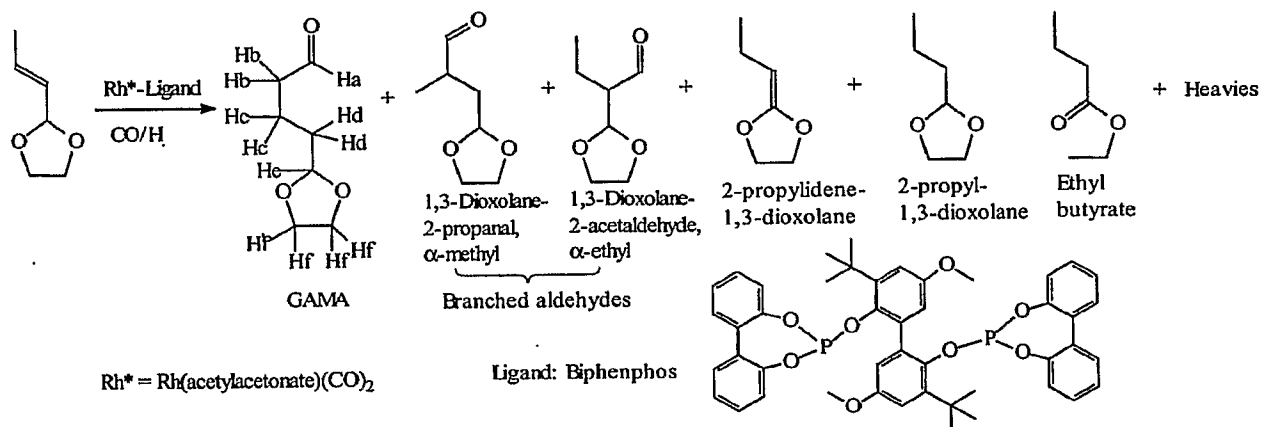
sodium or potassium cyanide and ammonium carbonate. In each of these alternative embodiments, the masked amino acid derivative is then subject to enantioselective catalytic bio-resolution. L-Selective amidases effective in the enantioselective hydrolysis of amino amide VIIa at about pH 9-10 are reported in US6174711 and US6291701, incorporated herein by reference. L-Selective hydantoinases, effective in the enantioselective hydrolysis of hydantoin VIIIa and typically used in conjunction with a carbamoylase or equivalent enzyme are reported in US6825014, incorporated herein by reference. None of the patents US6174711, US6291701 or US6825014 report hydroformylation as a process used to prepare bio-resolution substrates.



Although the preferred means of isolating the L-amino acid of formula IV in relatively pure form is by precipitation, it should be understood that other means of isolation can be used such as evaporation, chromatography and extraction.

EXAMPLE 1

The following reaction scheme (wherein Ha – Hf defines different substitution positions for hydrogen) summarizes the hydroformylation of 4-[1,3]dioxolan-2-yl-butyraldehyde to produce a product comprising a mixture of aldehydes and other products.



A hydroformylation catalyst solution is prepared in a glove box by dissolving 0.578 grams of Biphenphos (Ojima et al., J. Org. Chem., 1995, 60, 7078-7079) and 0.0978 grams of rhodium compound in 10 grams of tetrahydrofuran (THF) in a flask, which is then sealed with a septum. A feed of 93.04 grams of 1,3-dioxolane-2-butanal, 80 grams of THF and 8.09 grams of toluene is weighed out in the glove box. The catalyst solution is transferred into a 300 ml stirred Parr reactor under nitrogen. The nitrogen is purged from the reactor by pressurizing and venting 1:1 mole ratio CO:H₂ syn gas three times. The feed solution was transferred into a sample cylinder attached to the reactor and purged by pressurizing and venting 1:1 mole ratio CO:H₂ syn gas three times. The feed was added into the reactor when the reactor is heated to 85 degrees Celsius. The temperature of the reactor is controlled at 85 degrees Celsius. The pressure of syn gas in the reactor is controlled at 25 psig. After 7 hours, GC analysis showed 91% conversion of 4-[1,3]dioxolan-2-yl-butyraldehyde and 79% yield of GAMA.

About 75 milliliters of hexane is mixed with the reaction solution and this mixture is then extracted with degassed and deionized water (3x120 milliliters). The aqueous extract is then extracted with methylene chloride (5x120 milliliters). The methylene chloride extract is dried with MgSO₄ and filtered. After evaporating the methylene chloride, the neat product comprising the mixture of aldehydes is analyzed by GCMS and is found to contain GAMA (89.0wt%), branched aldehydes (4.7wt%) and heavies (2.6wt%). The final isolated yield for GAMA is about 70%.

Into a 3-neck 100 milliliter round bottom flask is placed 743 milligrams (13.9mmol) of ammonium chloride, 904 milligrams (13.9mmol) of potassium cyanide, 18 milliliters of water and 16 milliliters of 35% aqueous ammonia. The solution is stirred under a nitrogen

atmosphere and cooled to below zero degrees Celsius by means of a salt ice bath. To the cooled solution is slowly added over a ten minutes the above detailed product comprising the mixture of aldehydes, taking care that the temperature remains below 0 degrees Celsius. After the addition is complete, the reaction mixture is allowed to slowly warm up to room temperature and left stirring overnight. After overnight stirring the reaction is halted and extracted with 3x30 milliliters of ethyl acetate. The combined ethyl acetate extract is then dried over magnesium sulphate and the ethyl acetate evaporated under reduced pressure to yield a mixture comprising the acetal-aminonitriles.

Into a 25ml round bottom flask is placed 500 milligrams of the mixture comprising the acetal-aminonitriles in 1ml methanol and 9ml of distilled water. The pH of the stirred solution is adjusted to 7 by dropwise addition of 1M hydrochloric acid solution. Then 27 milligrams of nitrilase NIT-7478 (Diversa, lot 4556) is added. The reaction mixture is stirred for 43 hours at ambient temperature, during which time the pH is maintained between 7 and 8 by further dropwise additions of 1M hydrochloric acid solution. The reaction is then halted and the reaction mixture is concentrated to dryness under reduced pressure. The resulting residue is washed with 10 milliliters of ethyl acetate and the insoluble amino acid is recovered by filtration and dried in a vacuum oven to yield a white solid. Chiral HPLC analysis (penicillamine column) indicates an L-allysine ethylene acetal purity of 98.8% and an overall mole percent yield (based on moles of 1,3-dioxolane-2-butanal starting material) of about 30%.

EXAMPLE 2

This example starts with the aminonitrile mixture of Example 1. Into a 50ml round bottom flask is placed 725 milligrams of the aminonitrile mixture in 20 milliliters of 50% aqueous ethanol, together with 720 milligrams (18mmol, 4.2eq) of sodium hydroxide. The reaction mixture is stirred continuously and heated under refluxing conditions for 4 hours. After this time the mixture is cooled to below 5 degrees Celsius by means of a salt-ice bath and 490 microliters (5.11mmol, 1.2eq) of acetic anhydride is added to the stirred solution. The reaction mixture is allowed to warm up to room temperature and left stirring overnight. The mixture is then acidified to pH 3 with 6M HCl solution and extracted with 3x50 milliliters of ethyl acetate. The combined extracts are dried over magnesium sulphate and

the ethyl acetate is evaporated under reduced pressure to yield an orange oil containing about 500 milligrams of *N*-Ac allysine ethylene acetal as determined by GCMS.

Into a 50ml jacketed vessel is placed the above detailed orange oil comprising the *N*-Ac allysine ethylene acetal dissolved in 20ml of distilled water. The solution is continuously stirred, heated to 60 degrees Celsius and the pH of the solution is adjusted to 7 by dropwise addition of 1M NaOH solution. 1 milliliter (2650 units) of L-acylase solution (from *Thermococcus litoralis*, see Taylor *et al.* Biochem. Soc. Trans. (2004) 32(2), 290-292) is then added and the mixture is left stirring for 24 hours at 60 degrees Celsius. The reaction mixture is then acidified to pH 3 with 6M HCl solution and extracted with 3x25 milliliters of ethyl acetate in order to remove the residual *N*-Ac allysine ethylene acetal. The pH of the aqueous phase is readjusted to 7.3 using 5M sodium hydroxide solution, concentrated to one quarter volume and diluted with 20 milliliters of isopropanol. The resulting white precipitate is recovered by filtration and dried overnight in a vacuum oven, yielding 150 milligrams of L allysine ethylene acetal. Chiral HPLC analysis (penicillamine column) indicates an L-allysine ethylene acetal purity of 99%.

EXAMPLE 3

This example also starts with an aminonitrile mixture made according to the teachings of Example 1. Into a 250ml round bottom flask is placed ten grams of the aminonitrile mixture in 120ml of 50% aqueous ethanol, together with 10.54 grams (264mmol, 4eq) of sodium hydroxide. The reaction mixture is stirred continuously and heated under refluxing conditions for 5 hours. After this time the mixture is allowed to cool to room temperature and ethanol is removed under reduced pressure. The aqueous solution is then cooled to below 5 degrees Celsius by means of a salt-ice bath and a solution of 9.18 milliliters (79mmol, 1.2eq) of benzoyl chloride in 15ml THF is slowly added to the stirred solution. The reaction mixture is allowed to warm up to room temperature and left stirring overnight. After 20 hours, the mixture is acidified to pH 3 with 6M HCl solution and extracted with 3x75 milliliters of ethyl acetate. The combined extracts are dried over magnesium sulphate and concentrated under reduced pressure to yield an off white solid. This material is contaminated with benzoic acid and is purified by slurrying in methyl t-butyl ether (MTBE). The product comprises *N*-benzoyl allysine and is recovered by filtration and dried in a vacuum oven to yield an off white solid.

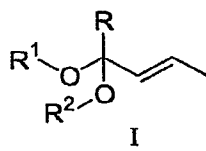
Into a 1 litre jacketed vessel is placed 48.4 grams of the above-detailed off white solid. The vessel was heated to 65°C and a solution of 470 milliliters of distilled water and 10 milliliters of 48% sodium hydroxide solution is added to the stirred vessel. The pH of the solution was adjusted to 7.5 by addition of a few drops of 48% NaOH solution. Twelve milliliters of L-acylase solution (31800Units) is then added and the mixture is left stirring for 18 hours at 65 degrees Celsius. The reaction mixture is filtered to remove any precipitated protein and the filtrate is concentrated under reduced pressure to approximately one third volume. The pH of the concentrated solution is adjusted to 7.4 using 5M sodium hydroxide solution and two volumes of ethanol were added. The mixture is cooled to below 10 degrees Celsius and left to stand for 1 hour. The precipitate is recovered by cold filtration, washed with further cold ethanol and dried overnight in a vacuum oven, yielding 12.3g of L-allysine ethylene acetal as a white crystalline solid. Chiral HPLC analysis (penicillamine column) indicates an L-allysine ethylene acetal purity of better than 99%.

CONCLUSION

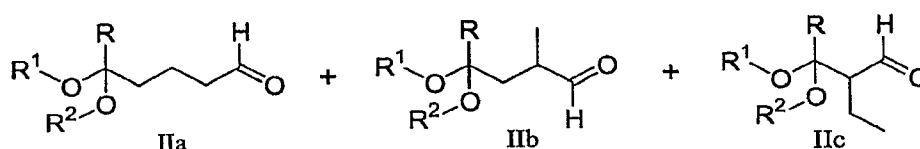
While the instant invention has been described above according to its preferred embodiments, it can be modified within the spirit and scope of this disclosure. This application is therefore intended to cover any variations, uses, or adaptations of the instant invention using the general principles disclosed herein. Further, the instant application is intended to cover such departures from the present disclosure as come within the known or customary practice in the art to which this invention pertains and which fall within the limits of the following claims.

WHAT IS CLAIMED IS:

1. A process for making an amino acid, comprising the steps of: (a) contacting a compound of formula I

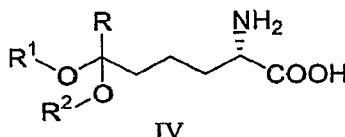


with a hydroformylation catalyst and synthesis gas to produce a mixture of aldehyde compounds comprising the formulas IIa, IIb and IIc;



(b) reacting the mixture of aldehyde compounds from step (a) to produce a mixture of derivative compounds comprising a masked amino acid derivative which may be selected from the group consisting of amino nitrile, N-acyl amino acid, amino amide, hydantoin and amino ester;

(c) contacting the mixture of derivative compounds from step (b) with an enantioselective hydrolase enzyme in the presence of water to produce an L-amino acid having the formula IV;

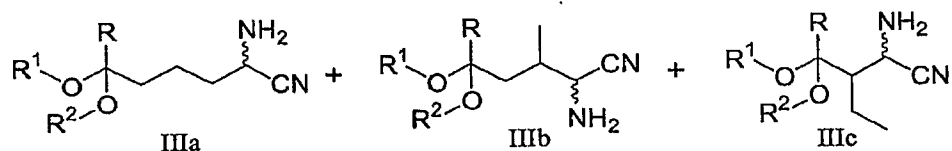


(d) isolating the amino acid having the formula IV in substantially pure form, wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc and IV, R is H, alkyl, aryl, arylalkyl, or alkylaryl and R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused.

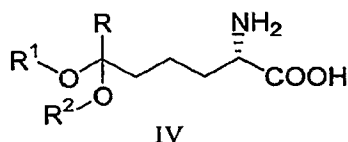
2. The process of Claim 1, wherein step (a) is carried out in an aprotic polar solvent, wherein between steps (a) and (b) a nonpolar solvent is added to the mixture of aldehyde compounds to form a mixture of aldehyde compounds in a mixture comprising the aprotic polar solvent and the nonpolar solvent which mixture is then extracted with water to form an aqueous phase comprising the mixture of aldehyde compounds and the aprotic polar solvent and an organic phase comprising the hydroformylation catalyst and the nonpolar

solvent so that the hydroformylation catalyst can be separated from the mixture of aldehyde compounds.

3. The process of Claim 2, wherein the aprotic polar solvent comprises tetrahydrofuran.
4. The process of Claim 2 or 3, wherein the nonpolar solvent comprises an alkane.
5. The process of Claim 4, wherein the nonpolar solvent comprises hexane.
6. The process of any one of Claims 2-5, wherein the aprotic polar solvent consists essentially of tetrahydrofuran and wherein the nonpolar solvent consists essentially of an alkane.
7. The process of Claim 6, wherein the nonpolar solvent consists essentially of hexane.
8. The process of any one of Claims 1-7, wherein the compound of formula I is 4-[1,3]dioxolan-2-yl-butyraldehyde and wherein the compound of formula IV is L-allysine ethylene acetal.
9. The process of any one of Claims 1-8, wherein the hydroformylation catalyst comprises a rhodium complex of the ligand Biphephos.
10. The process of any one of Claims 2-8, wherein the aprotic polar solvent has an aqueous-hexane partition coefficient in the range of from 8:1 to 1:2.
11. The process of Claim 1, wherein the mixture of derivative compounds of step (b) comprises a masked amino acid derivative selected from the group consisting of amino nitrile, N-acyl amino acid, amino amide, hydantoin and amino ester.
12. The process of Claim 1, wherein step (b) comprises contacting the mixture of aldehyde compounds with a Strecker reagent to produce a mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc; and

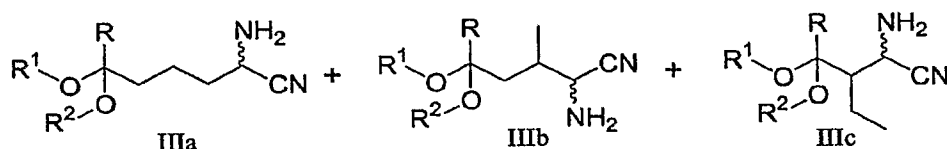


step (c) comprises contacting the mixture of nitrile compounds with an L-specific nitrilase to produce an L-amino acid having the formula IV,

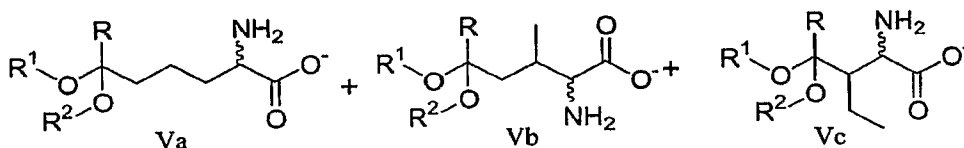


wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc and IV, R is H, alkyl, aryl, arylalkyl, or alkylaryl, and R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused.

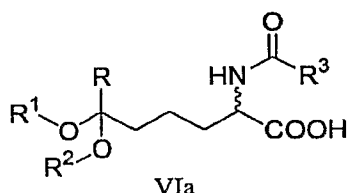
13. A process according to claim 1, wherein step (b) comprises contacting the mixture of aldehyde compounds with a Strecker reagent to produce a mixture of nitrile compounds comprising the formulas IIIa, IIIb and IIIc;



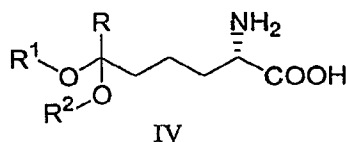
and further comprises contacting the mixture of nitrile compounds with aqueous ethanol under basic conditions to produce a mixture of amino acid salts comprising the formulas Va, Vb and Vc;



and further comprises contacting the mixture of amino acid salts with an acylating reagent to produce an acetal compound comprising the formula VIa; and

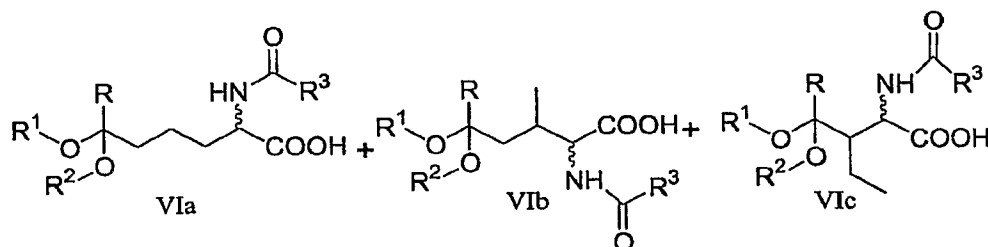


step (c) comprises contacting the acetal compound with an L-specific aminoacylase to produce an L-amino acid having the formula IV,



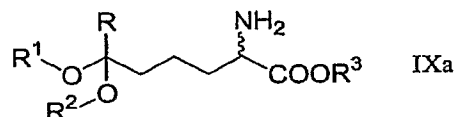
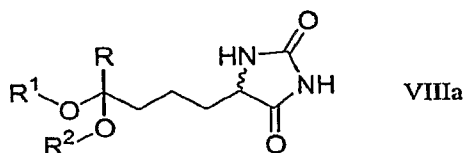
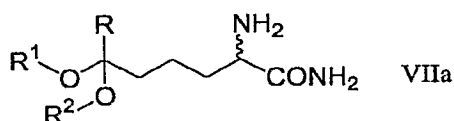
wherein in formulas I, IIa, IIb, IIc, IIIa, IIIb, IIIc, Va, Vb, Vc, VIa and IV, R is H, alkyl, aryl, alkylaryl, or arylalkyl, wherein R³ is an alkyl, aryl or alkylaryl group containing from one to ten carbons and wherein R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused.

14. The process of Claim 13, wherein in step (b) a mixture of acetal compounds is produced comprising the formula VIa, VIb and VIc;



wherein in step (c) the mixture of acetal compounds is contacted with the L-specific N-acylase to produce the L-amino acid having the formula IV, and wherein in formulas VIb and VIc R is H, alkyl, aryl, arylalkyl, or alkylaryl, wherein R³ is an alkyl, aryl or alkaryl group containing from one to ten carbons and wherein R¹ and R² are the same or different alkyl groups and wherein R¹ and R² may be fused.

15. The process of Claim 2, wherein the mixture of derivative compounds of step (b) comprises a compound of formula VIIa, VIIIa or IXa



where R is H, alkyl, aryl, arylalkyl, or alkylaryl, and R¹ and R² are the same or different alkyl groups wherein R¹ and R² may be fused and where R³ is an alkyl or aralkyl group containing from one to ten carbons.

16. The process of any one of the preceding claims, wherein the enantioselective hydrolase enzyme of step (c) is selected from the group consisting of nitrilases, nitrilase hydratases, aminoacylases, amidases, hydantionases, and esterases.

17. The process of any one of the preceding claims, wherein the isolation of step (d) comprises precipitation.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/001207

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C227/32 C07D317/30 C12P41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 972 845 A1 (MITSUBISHI GAS CHEMICAL CO [JP]) 19 January 2000 (2000-01-19) claims 3,4 page 3, line 43 - line 44	1
A	CUNY G D ET AL: "PRACTICAL, HIGH-YIELD, REGIOSELECTIVE, RHODIUM-CATALYZED HYDROFORMYLATION OF FUNCTIONALIZED ALPHA-OLEFINS" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US, vol. 115, no. 5, 10 March 1993 (1993-03-10), pages 2066-2068, XP000604731 ISSN: 0002-7863 cited in the application page 2067, table 1, entry 13	1
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

9 May 2007

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/001207

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 03/078444 A (UNION CARBIDE CHEM PLASTIC [US]; PENG WEI-JUN [US]; BRYANT DAVID ROBER) 25 September 2003 (2003-09-25) cited in the application claim 30 page 3, line 2 - line 14 -----	1
A	US 4 668 651 A (BILLIG ERNST [US] ET AL) 26 May 1987 (1987-05-26) cited in the application column 19, line 24 - line 65 column 29 - column 36 -----	1
A	WO 02/10424 A (DEGUSSA [DE]) 7 February 2002 (2002-02-07) claim 1 -----	1
A	US 2004/002147 A1 (DESANTIS GRACE [US] ET AL) 1 January 2004 (2004-01-01) cited in the application page 77, column 1, last paragraph - column 2, paragraph 1 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/001207

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0972845	A1	19-01-2000	AT 232240 T 15-02-2003
		DE 69905218 D1 13-03-2003	
		DE 69905218 T2 22-01-2004	
		US 6174711 B1 16-01-2001	
WO 03078444	A	25-09-2003	AT 320438 T 15-04-2006
		AU 2003230587 A1 29-09-2003	
		CN 1639177 A 13-07-2005	
		DE 60304034 T2 12-10-2006	
		EP 1485392 A2 15-12-2004	
		JP 2005519968 T 07-07-2005	
		US 2006100453 A1 11-05-2006	
US 4668651	A	26-05-1987	AR 242182 A1 31-03-1993
		AU 597593 B2 07-06-1990	
		AU 6237386 A 12-03-1987	
		BR 8604261 A 05-05-1987	
		CA 1281704 C 19-03-1991	
		CN 86106811 A 29-04-1987	
		CN 1041761 A 02-05-1990	
		CS 8606430 A3 19-02-1992	
		CS 8807490 A3 19-02-1992	
		DE 3685276 D1 17-06-1992	
		DK 423486 A 06-03-1987	
		EP 0214622 A2 18-03-1987	
		ES 2001416 A6 16-05-1988	
		FI 863570 A 06-03-1987	
		HU 46642 A2 28-11-1988	
		IN 168034 A1 26-01-1991	
		JP 1765977 C 11-06-1993	
		JP 4051531 B 19-08-1992	
		JP 62116535 A 28-05-1987	
		MX 164384 B 10-08-1992	
		NO 863546 A 06-03-1987	
		PL 261286 A1 14-12-1987	
		YU 155086 A1 29-02-1988	
		ZA 8606728 A 29-04-1987	
WO 0210424	A	07-02-2002	AR 029840 A1 16-07-2003
		AU 6910401 A 13-02-2002	
		DE 10037115 A1 07-02-2002	
		EP 1305441 A1 02-05-2003	
		US 2002132848 A1 19-09-2002	
US 2004002147	A1	01-01-2004	NONE