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(54) Benævnelse: **Anvendelse af precursorer til fremstilling af carbon-11-mærkede aminosyrer og derivater deraf**

(56) Fremdragne publikationer:
**JUNHAO XING ET AL: "High-Yielding Automated Convergent Synthesis of No-Carrier-Added [11C-Carbonyl]-Labeled Amino Acids Using the Strecker Reaction", SYNLETT, Bd. 28, Nr. 03, 7. November 2016 (2016-11-07), Seiten 371-375, XP055483195, DE ISSN: 0936-5214, DOI: 10.1055/s-0036-1588638 in der Anmeldung erwähnt**
**PRASAD B A B ET AL: "Trimethylsilyl cyanide addition to aldimines and its application in the synthesis of (S)-phenylglycine methyl ester", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM, NL, Bd. 45, Nr. 52, 20. Dezember 2004 (2004-12-20), Seiten 9565-9567, XP027297918, ISSN: 0040-4039 [gefunden am 2004-11-26]**

**Use of precursors for the preparation of carbon-11 labelled amino acids and derivatives thereof**

The invention relates to the use of precursors for the preparation of carbon-11 labelled amino acids and amino acid derivatives as well as a method for the preparation of carbon-11 labelled amino acids and amino acid derivatives using said precursors.

Carbon-11 radio-labelled compounds are used as radiopharmaceuticals in the positron emission tomography (PET). Here, they can be employed in the oncologic imaging as tracers. They are also used in the neuro-oncology. An important group of these radio-labelled compounds are carbon-11 radio-labelled amino acids. These amino acids belong to the chiral PET radiopharmaceuticals. For example, they can be used to represent the increased amino acid metabolism that is described for cancer cells. Radioactive labelling of amino acids with carbon-11 has the advantage that the radio-labelled amino acid and the endogenous molecule have an identical structure. Compared to fluoride-18-labelled amino acids the effort to be able to employ carbon-11-labelled amino acids in the clinical practice is significantly lower, because the pharmacological and toxicological tests each needed for that require less effort. This fact, for example, shows in the comparison of [$^{11}$C]choline that is a carbon-11 labelled variant of the endogenous compound choline and its fluorinated analogue, [$^{16}$F]fluorocholine.

Many carbon-11 amino acid isotopologues have been prepared with varying successes and often in an asymmetric manner in which carbon-12 is substituted with radioactive carbon-11. The greatest challenge in the synthesis of carbon-11 labelled amino acids still is the stereo-selective reaction at the a-carbon to prevent time-wasting chiral separation via HPLC to obtain the enantiomerically pure form simply and reliably from the radiolabeling process which itself is limited by the synthesis time and the specific activity.

The importance of the enantiomeric purity of radiopharmaceuticals is of great importance for radioactive labelled amino acids since the stereochemistry has influence on the rate and selectivity of the amino acid transport. For this reason, L-enantiomers are preferred in mammalian cells as could be shown by the use of L- und D-[$^{11}$C]phenylalanine, wherein the L-enantiomer of [$^{11}$C]phenylalanine exhibited better pancreas-to-liver-ratios.

This was also proved by the better imaging properties of L-$^{18}$F-labelled fluoroalkyl-phenylalanine analogues all exhibiting a high tumor intake compared to surrounding tissue. Therefore, these studies - without elaborating on the gold standards L-[$^{11}$C]methionine and L-[$^{18}$F]fluoroethyltyrosine - underline the necessity to develop methods for the synthesis of enantiomerically pure amino acids as PET tracers.

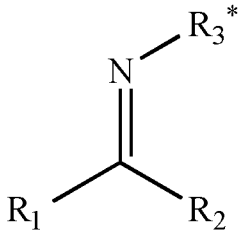
Moreover, the half-life of carbon-11 (20.39 min.) allows that examinations with different [$^{11}$C]-labelled PET radiotracers can be performed on a patient on the same day during a single hospital visit. However, the half-life also means that the period of time that is available for the preparation and use of the [$^{11}$C]-labelled PET radiotracer is extremely short. Hence, time-consuming syntheses significantly lower the practical applicability of a [$^{11}$C]-labelled PET radiotracer. Generally, only 60 min. are available for the synthesis, purification, and quality control of a [$^{11}$C]-labelled PET radiotracer. That is, the number of synthesis steps has to be as low as possible. Additionally, the reaction time required for one synthesis step has to be as short as possible.

For the synthesis of carbon-11 labelled amino acids the Strecker reaction can be applied (see, Xing, J. et al., High-yielding automated convergent synthesis of no-carrier-added [11C-carbonyl]-labeled amino acids using the strecker reaction. *Synlett* (2017), *28*(3), 371-375). The variant of the Strecker reaction for the preparation of [$^{11}$C]sacrosine suggested by Xing et al., wherein the carbon atom of the carbonyl group is the carbon-11 atom requires the preparation of [$^{11}$C]□-aminonitrile by the condensation of formaldehyde with methylamine and [$^{11}$C]sodium cyanide. The aminonitrile thus obtained subsequently is subjected to a basic hydrolysis with sodium hydroxide. Said variant can be adopted by other [$^{11}$C]amino acids, wherein the formaldehyde has to be replaced by another ketone or the methylamine by another amine or both compounds. The required synthesis times are under 20 min. However, the radioactive yields are low. Moreover, in this method it is not possible to enantioselectively prepare amino acids. Prasad, B., et al. (Tetrahedron Letters (2004) 45, 9565-9567) describe the addition of tri-methylsilylcyanide to arylaldimines as a form of the Strecker synthesis.

The problem of the invention is to eliminate the drawbacks according to the prior art. In particular, use of precursors for the enantioselective preparation of amino acids and derivatives thereof shall be suggested. Furthermore, a method for the diastereoselective labelling of said precursors with carbon-11 shall be suggested.

This problem is solved by the features of claims 1 and 5. Suitable developments of the inventions result from the features of the dependent claims.

According to the invention there is provided the use of a precursor for the preparation of carbon-11 labelled amino acids or derivatives. The precursor is a compound of formula I:




(formula I)

wherein

$R_1$ and $R_2$ are independently selected from the group comprising hydrogen, unsubstituted or substituted $C_1$-$C_6$alkyl that can optionally be modified by inserting at least one group X into the carbon chain, unsubstituted or substituted $C_2$-$C_6$alkenyl that can optionally be modified by inserting at least one group X into the carbon chain, substituted or unsubstituted alkyl aryl, substituted or unsubstituted aryl alkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl, wherein $R_1$ is preferably unsubstituted or substituted $C_1$-$C_6$alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl, more preferred substituted or unsubstituted $C_1$-$C_6$alkyl, or substituted or unsubstituted aryl, and particularly preferred unsubstituted or substituted $C_1$-$C_6$alkyl, and $R_2$ is preferably unsubstituted or substituted $C_1$-$C_6$alkyl, or hydrogen, particularly preferred hydrogen,

$R_3$ is a chiral auxiliary selected from the group comprising substituted or unsubstituted $C_1$-$C_6$alkyl sulphinyl, substituted or unsubstituted aryl sulphinyl, substituted or unsubstituted aryl alkyl, and substituted or unsubstituted aryl glycinol, wherein $R_3$ is preferably substituted or unsubstituted $C_1$-$C_6$alkyl sulphinyl,

X is selected from the group comprising oxygen, sulphur, -SO-, -$SO_2$-, and -N($R_{10}$)-,

$R_{10}$ comprises hydrogen, unsubstituted or substituted $C_1$-$C_6$alkyl, unsubstituted or substituted $C_2$-$C_6$alkenyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and

the residues are optionally unprotected or protected. In particular $R_1$ and/or $R_2$ can be unprotected or protected, and the other residues can be unprotected.

The precursor according to the invention permits the diastereoselective labelling with a carbon-11 synthon, i.e., there is preferably obtained a diastereomer. By the subsequent cleavage of the chiral auxiliary the precursor thus permits the enantioselective preparation of carbon-11 labelled amino acids and derivatives thereof. In particular, the precursor according to the invention can be used for the enantioselective preparation of α-amino acids and derivatives thereof such as α-amino acid esters and α-amino acid amides, particularly preferred L-α-amino acids and derivatives thereof such as L-α-amino acid esters and L-α-amino acid amides. However, the precursor according to the invention can also be used for the enantioselective preparation of D-α-amino acids and derivatives thereof such as D-α-amino acid esters and D-α-amino acid amides. Thus, the precursor according to the invention permits the enantioselective preparation either of the L-α-amino acids and derivatives thereof such as L-α-amino acid esters and L-α-amino acid amides or the D-α-amino acids and derivatives thereof such as D-α-amino acid esters and D-α-amino acid amides.

The precursors of formula I should be thermodynamically stable compounds.

In the present invention the term “substituted” particularly relates to one or more substituents selected from the group comprising halogen, cyano, nitro, protected or unprotected hydroxy, protected or unprotected -N($R_{11}R_{12}$) and protected or unprotected thiol. $R_{11}$ and $R_{12}$ independently may be selected from the group comprising hydrogen, unsubstituted or substituted $C_1$-$C_6$alkyl, unsubstituted or substituted $C_2$-$C_6$alkenyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. Preferably, $R_{11}$ and $R_{12}$ both are hydrogen.

The term “protected” particularly relates to a protecting group for the protection of the hydroxy group, the -N($R_{11}R_{12}$) group or the thiol group. The protecting group can be removed after the reaction of the precursor. For the protection of the hydroxy group known protecting groups can be used. A protecting group for the protection of the hydroxy group may for example be selected from the group comprising trityl, benzyl, methoxybenzyl, p-nitrobenzyl, benzoyl, substituted benzoyl, trimethylsilyl, triethylsilyl, isopropyldimethylsilyl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl, thexyldimethylsilyl,

allyl, methoxymethyl, (2-methoxyethoxy)methyl, and tetrahydropyranyl. For the protection of the amine group, i.e. $R_{11}$ and $R_{12}$ both are hydrogen, known protecting groups can be used. A protecting group for the protection of the amine group may for example be selected from the group comprising tert-butylcarbonyl, benzylcarbonyl, 9-fluorenylmethylcarbonyl, and allylcarbonyl. For the protection of the thiol group known protecting groups can be used.

The term “halogen” relates to fluorine, chlorine, bromine, or iodine.

The term “alkyl” particularly relates to a saturated aliphatic hydrocarbon group having a branched or unbranched carbon chain with 1 to 6 carbon atoms.

The term “alkylene” particularly relates to an unsaturated aliphatic hydrocarbon group with 2 to 6 carbon atoms, for example ethylene, 2,2-dimethylethylene, propylene, 2-methylpropylene, butylene, pentylene, and the like.

The term “aryl” relates to a monovalent cyclic aromatic hydrocarbon group which can comprise a mono-, bi- or tricyclic aromatic ring. The aryl group can optionally be substituted. Examples of aryl groups are – optionally substituted – phenyl, naphthyl, phenanthryl, fluorenyl, indenyl, azulenyl, oxydiphenyl, biphenyl, methylenediphenyl, aminodiphenyl, diphenylsulfidyl, diphenylsulfonyl, diphenylisopropylidenyl, benzodioxanyl, benzodioxylyl, benzoxazinyl, benzoxazinonyl, benzopiperadinyl, benzopiperazinyl, benzopyrrolidinyl, benzomorpholinyl, methylenedioxyphenyl, ethylenedioxyphenyl, and the like, wherein the list is not complete. Preferably, aryl comprises optionally substituted phenyl and optionally substituted naphthyl.

The term “heteroaryl” particularly relates to a monocyclic, bicyclic, or tricyclic group with 5 to 12 ring atoms, wherein at least one aromatic ring contains one, two, or three ring heteroatoms selected from N, O or S, wherein the remaining ring atoms are C. The heteroaryl group can optionally be substituted. Examples of heteroaryl groups are – optionally substituted – imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, pyrazinyl, pyridazinyl, thiophenyl, furanyl, pyranyl, pyridinyl, pyrrolyl, pyrazolyl, pyrimidyl, quinolinyl, isoquinolinyl, quinazolinyl, benzofuranyl, benzothiophenyl, benzothiopyranyl, benzimidazolyl, benzoxazolyl, benzooxadiazolyl, benzothiazolyl, benzothiadiazolyl, benzopyranyl, indolyl, isoindolyl, indazolyl, triazolyl,

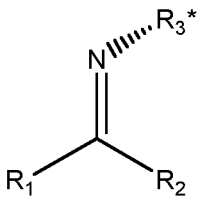
triazinyl, quinoxalinyl, purinyl, quinazolinyl, quinolizinyl, naphthyridinyl, pteridinyl, carbazolyl, azepinyl, diazepinyl, acridinyl and the like, wherein the listing is not complete.

The term "alkylaryl" particularly relates to monovalent alkyl residues bearing an aryl group, wherein the alkyl residue can have 1 to 6 carbon atoms, as defined above.

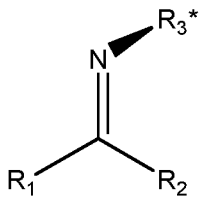
The term "arylalkyl" particularly relates to monovalent aryl residues bearing an alkyl group, wherein the alkyl residue can have 1 to 6 carbon atoms, as defined above.

Preferably, $R_1$ is selected from the group comprising methyl, protected and unprotected hydroxymethyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butanyl, sec-butyl, tert-butyl, phenyl, protected and unprotected hydroxyphenyl, benzyl, and protected and unprotected hydroxybenzyl, and $R_2$ is hydrogen.

The term "chiral auxiliary" particularly relates to a group having at least one chiral center. The chiral auxiliary permits a diastereoselective reaction of the precursor according to the invention such that preferably an enantiomer is obtained after the cleavage of the chiral auxiliary. Preferred chiral auxiliaries are substituted or unsubstituted alkylsulfinyl and substituted or unsubstituted arylsulfinyl, wherein substituted or unsubstituted alkylsulfinyl is preferred. Due to the chiral auxiliary the precursor of formula I has two enantiomers. The two enantiomers are illustrated in formulae I-a and Ib, wherein the representation of the binding between the nitrogen atom and group $R_3$ shall only illustrate the two enantiomers, but does not mandatory correspond to the projection rules, because these depend on the structure of residue $R_3$. Formula I-a illustrates an enantiomer that can preferably be used for the preparation of the D-amino acid. Formula I-b illustrates an enantiomer that can preferably be used for the preparation of the L-amino acid:



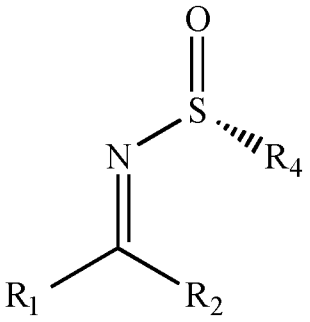

(formula I-a)



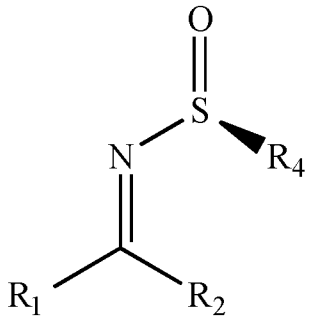

(formula I-b)

wherein $R_1$, $R_2$, and $R_3$ are as defined above. Depending on the chiral auxiliary and reaction condition either the L- or D-amino acid is preferably formed.

The chiral auxiliaries referred to as alkylsulfinyl and arylsulfinyl represent a -S(O)-$R_4$ group, wherein $R_4$ is selected from the group comprising unsubstituted or substituted $C_1$-$C_6$alkyl and substituted or unsubstituted aryl. In this case the compound of formula I is a compound of formula II-a or II-b:



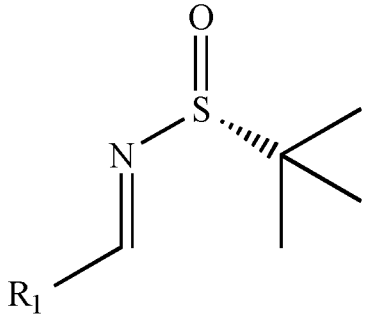
(formula II-a)

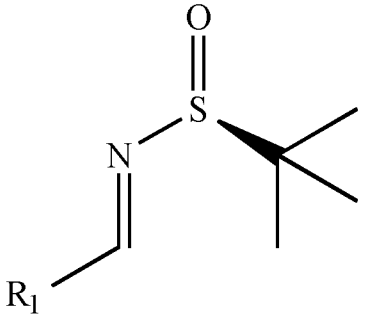


(formula II-b)

wherein $R_1$ and $R_2$ are as defined above and $R_4$ is selected from the group comprising unsubstituted or substituted $C_1$-$C_6$alkyl and substituted or unsubstituted aryl. Preferably, $R_4$ is *tert.*-butyl. For the preparation of L-amino acids and their derivatives preferably use is made of a compound of formula II-b.

In the preferred embodiment the precursor according to the invention is a compound of formula II-a or II-b, wherein $R_1$ is selected from the group comprising unsubstituted or substituted $C_1$-$C_6$alkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; $R_2$ is hydrogen; and $R_4$ is selected from the group comprising unsubstituted or substituted $C_1$-$C_6$alkyl and substituted or unsubstituted aryl. In a more preferred embodiment, the precursor according to the invention is a compound of formula II-a or II-b, wherein $R_1$ is selected from the group comprising unsubstituted or substituted $C_1$-$C_6$alkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; $R_2$ is hydrogen; and $R_4$ is *tert.*-butyl. Such a precursor is a compound of formula III-a or III-b:



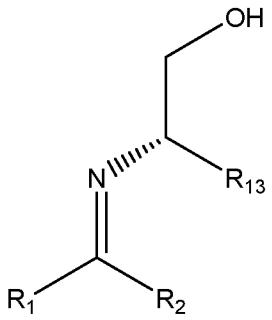


(formula III-a) (formula III-b)

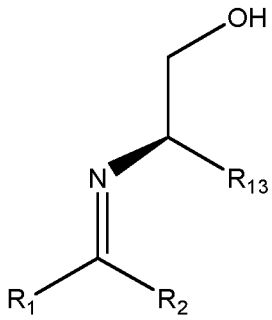
In a more preferred embodiment, the precursor according to the invention is a compound of formula III-a or III-b, wherein $R_1$ is selected from the group comprising methyl, protected and unprotected hydroxymethyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butanyl, sec-butyl, tert-butyl, phenyl, protected and unprotected hydroxyphenyl, benzyl, and protected and unprotected hydroxybenzyl.

The chiral auxiliaries referred to as "arylalkyl" represent an aryl group bearing one or more $C_1$-$C_6$alkyl groups. The arylalkyl group can be substituted or unsubstituted. Examples are – optionally substituted – benzyl, phenylethyl, and arylglycinol, wherein the listing is not complete.

The chiral auxiliaries referred to as "arylglycinol" represent a subgroup of chiral auxiliaries referred to as "arylalkyl". For example, precursors having an arylglycinol group may be a group of formula IV-a or IV-b:




(formula IV-a)




(formula IV-b)

wherein $R_{13}$ is substituted or unsubstituted aryl, as defined above. Examples of an arylglycinol group are – optionally substituted or unsubstituted – phenylglycinol, protected or unprotected phenylglycinol, wherein the listing is not complete.

The precursors according to the invention allow a rapid and diastereoselective reaction with a carbon-11 synthon. Said reaction, also referred to as labelling reaction, is diastereoselective. Due to the subsequent cleaving-off of the chiral auxiliary, for example by alcoholysis and/or hydrolysis, an enantiomer is formed in excess. That means, that as a result the method according to the invention is enantioselective.

In particular, the precursors according to the invention can be used to prepare amino acids, particularly α-amino acids and derivatives thereof such as amino acid esters and amino acid amides, the carbonyl moiety of which has a carbon-11 atom instead of a carbon-12 atom. The remaining carbon atoms of the amino acids and amino acid derivatives preferably are no carbon-11 atoms. The time required for the synthesis of said compounds is less than 20 min. The radiochemical yield is more than 10% (not decomposition corrected (n.d.c.)) and more than 20% (decomposition corrected (d.c.), respectively, each with respect to formed [$^{11}$C]HCN).

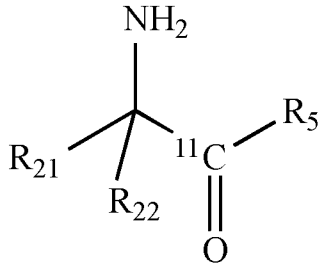
The precursors according to the invention may be synthesized for example by condensation of the corresponding aldehyde with an alkylsulfinyl amine in the presence of anhydrous copper sulphate in dichloromethane at room temperature and optionally subsequently chromatographically purified.

Further, in accordance to the invention a method for the diastereoselective labelling of a precursor according to the invention with carbon-11 is provided, wherein the precursor is reacted with a carbon-11 labelled synthon to a carbon-11 labelled compound having a carbon-11 labelled carbonyl group. Preferably, the carbon-11 labelled synthon is [$^{11}$C]$R_{31}$CN, wherein $R_{31}$ is selected from the group comprising hydrogen, an alkaline metal such as lithium, sodium, or potassium, acetyl, and alkyl silyl. Preferably, $R_{31}$ is hydrogen, sodium, or potassium, particularly preferred hydrogen or an alkyl silyl, particularly preferred hydrogen. A preferred example of an alkyl silyl is trimethyl silyl. $R_{31}$ may be present as a cation to the anion [$^{11}$C]$CN^-$. [$^{11}$C]$R_{31}$CN is preferably passed through the reaction mixture containing the precursor and optionally solvents and excipients as a gas stream. [$^{11}$C]$R_{31}$CN is preferably prepared by conversion from [$^{11}$C]$CO_2$. This may be done by means of known methods. The reaction of the precursor with the synthon is not a specific reaction. Rather, it depends on the chiral auxiliary and/or the reaction conditions, especially on the excipient(s) added and/or the solvent(s) employed which of both enantiomers is obtained in the reaction of the precursor with the synthon. Hence, said reaction is not a specific reaction.

The carbon-11 labelled compound may for example be an amino acid and derivatives thereof such as amino acid esters and amino acid amides, preferably α-amino acids and derivatives thereof such as α-amino acid esters and α-amino acid amides, particularly preferred L-α-amino acids and derivatives thereof such as L-α-amino acid esters and L-

α-amino acid amides. However, the carbon-11 labelled compound may also be D-α-amino acids and derivatives thereof such as D-α-amino acid esters and D-α-amino acid amides. The method according to the invention permits the enantioselective preparation either of the L-α-amino acids and derivatives thereof such as L-α-amino acid esters and L-α-amino acid amides, or the D-α-amino acids and derivatives thereof such as D-α-amino acid esters and D-α-amino acid amides.

For example, the carbon-11 labelled compounds may be compounds of formula X:




(formula X)

wherein

$R_{21}$ and $R_{22}$ have the meaning given above in connection with the residues $R_1$ and $R_2$ of the precursor according to the invention,

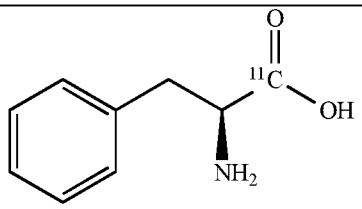
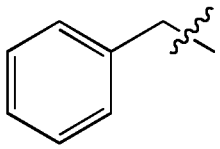
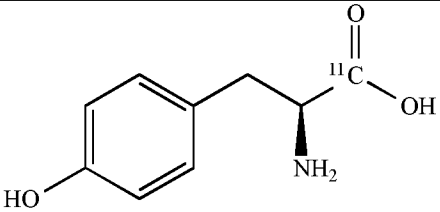
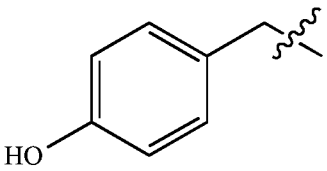
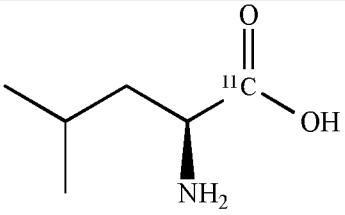
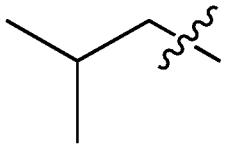
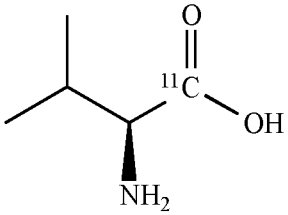
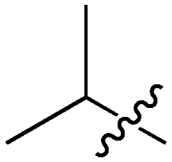
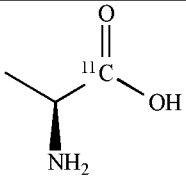
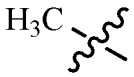
$R_5$ is selected from the group comprising $OR_6$ and $NR_7R_8$, and

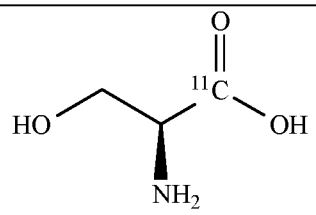
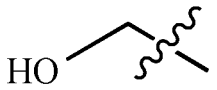
$R_6$, $R_7$, and $R_8$ are independently selected from the group comprising hydrogen, unsubstituted or substituted $C_1$-$C_6$alkyl, unsubstituted or substituted $C_2$-$C_6$alkenyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. The terms “alkyl”, “alkenyl”, “aryl”, “heteroaryl”, and “substituted” may have the meanings given above in connection with the precursor according to the invention. $R_7$ and $R_8$ preferably are hydrogen.

For the preparation of a compound of formula X that shall have certain residues $R_{21}$ and $R_{22}$ it is suitably selected a precursor which – apart from optionally provided protecting groups on the residues $R_1$ and/or $R_2$ – has residues $R_1$ and $R_2$ that correspond to the residues $R_{21}$ and $R_{22}$ of the compound of formula X. When using a precursor having protecting groups on the residues $R_1$ and/or $R_2$, then the method according to the invention can include the cleaving-off of the protecting groups.

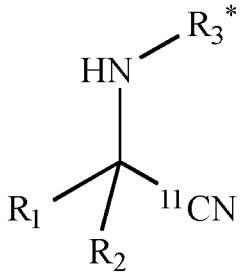
Examples of compounds of formula X are given in table 1 below, wherein the listing is not complete. In these compounds $R_2$ each is hydrogen and $R_5$ is hydroxy.

Table 1

| Formula No. | Compound | $R_1$ |
|---|---|---|
| X-11 |  ([1-$^{11}$C]-L-phenylalanine) |  (benzyl) |
| X-12 |  ([1-$^{11}$C]-L-tyrosine) |  (para-hydroxyphenylbenzyl) |
| X-13 |  ([1-$^{11}$C]-L-leucine) |  (iso-butyl) |
| X-14 |  [1-$^{11}$C]-L-valine |  (iso-propyl) |
| X-15 |  [1-$^{11}$C]-L-alanine |  (methyl) |
| | | |

| Formula No. | Compound | $R_1$ |
|---|---|---|
| X-16 |  [1-$^{11}$C]-L-serine |  (hydroxymethyl) |

The method according to the invention can include the reaction of the precursor according to the invention with the carbon-11 labelled synthon to a compound of formula V:

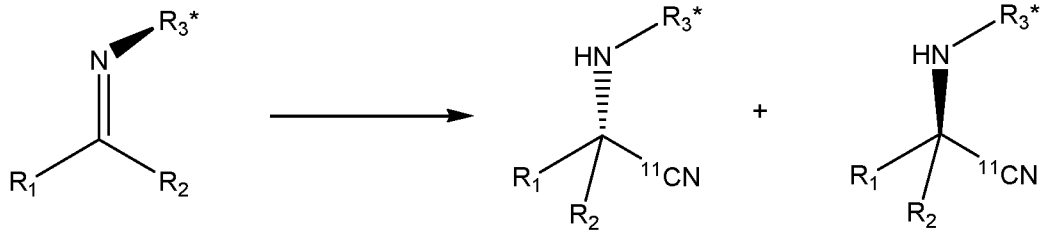


(formula V)

wherein $R_1$, $R_2$, and $R_3$ have the meanings given in claim 1. The reaction conditions are preferably selected such that the carbon-11 labelled synthon binds to the precursor by nucleophilic addition. Preferably, the reaction takes place in an aprotic solvent, for example 1,4-dioxane, tetrahydrofurane, diethylether, methyl-tert-butylether, toluene, benzol, dichloromethane, and other halogenated solvents, wherein the listing is not complete, a protic organic solvent, for example methanol, ethanol, iso-propanol, n-propanol, n-butanol, or the solvents water, DMSO, and DMF, wherein the listing is not complete, or mixtures of an aprotic solvent and a protic organic solvent. A solvent mixture is preferred that has a blending ratio of the aprotic solvent and the protic organic solvent in any conceivable ratio. Particularly preferred is a blending ratio of 8 to 2 based on the volume. A particularly preferred solvent mixture is a mixture of 1,4-dioxane and methanol. Preferably, the reaction is carried out at a temperature of 10 to 60°C, particularly preferred at room temperature. The reaction time may be between 1 and 10 min. The reaction mixture in addition to the precursor, the carbon-11 labelled synthon, and the solvent can contain one or more excipients. Such an excipient may be an addition salt such as cesium fluoride, “tetrabutylammoniumfluoride” (TBAF), tin diiodide,

aluminum trichloride, or other Lewis acids or fluoride salts, wherein cesium fluoride is preferred. When using [$^{11}$C]HCN as the synthon, then the labelling yields are 70 to 95% with respect to the [$^{11}$C]HCN employed.

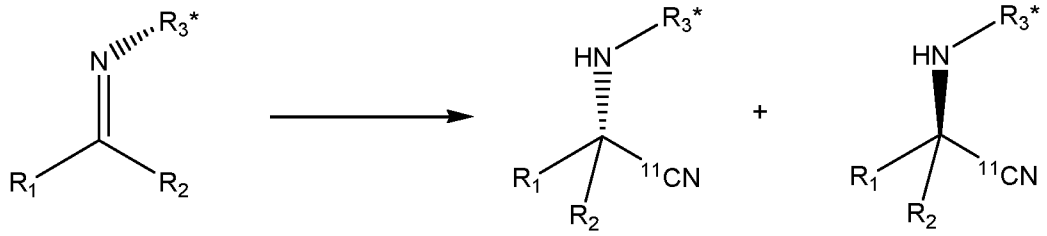
Preferably, a compound of formula I-b is reacted to a compound of formula V-a (scheme 1a):




I-b V-a V-b

scheme 1a

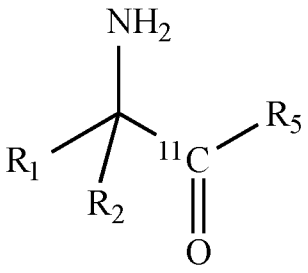
The reaction according to scheme 2a is particularly advantageous if the method according to the invention is to be employed for the preparation of L-amino acids and their derivatives. It depends on the precursor, especially the chiral auxiliary of the precursor employed, and the reaction conditions, especially the excipient(s) and the solvent(s) which of the two compounds V-a or V-b is obtained in excess. Alternatively, a compound of formula I-a can be reacted to a compound of formula V-a or V-b (scheme 1b). It depends on the precursor, especially the chiral auxiliary of the precursor employed, and the reaction conditions, especially the excipient(s) and the solvent(s) which of the two compounds V-a or V-b is obtained in excess. The reaction of precursor I with the synthon is not a specific reaction.




I-a V-a V-b

scheme 1b

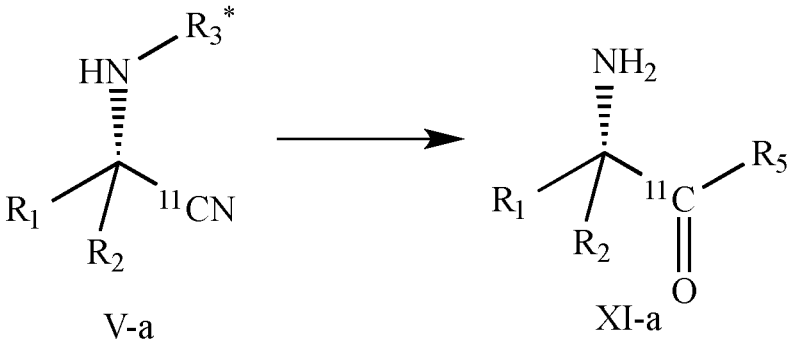
In the next step the compound of formula V can be converted to a compound of formula XI by means of alcoholysis or hydrolysis (alcoholysis results in $OR_6$, hydrolysis results in $NR_7R_8$ or OH), wherein the alcoholysis results in $R_5 = OR_6$ and the hydrolysis results in $R_5 = NR_7R_8$ or OH:



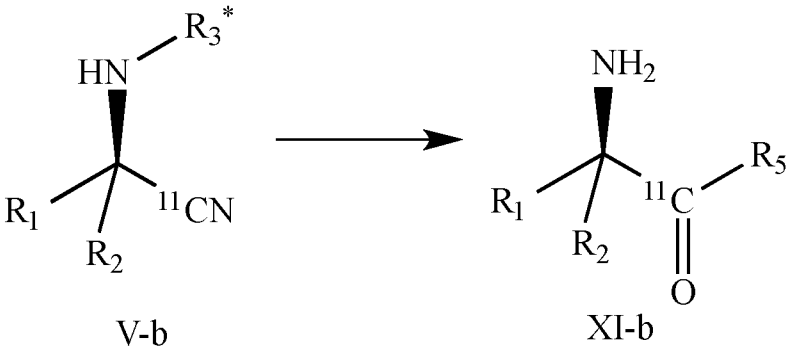
(formula XI)

wherein $R_1$, $R_2$, and $R_5$ are as defined above, with the provision that $R_5$ is not OH (i.e., $R_6$ is not hydrogen). The compound of formula XI comprises no amino acids. Apart from that and provided that the precursor has no protecting group, the compound of formula XI corresponds to the compound of formula X. That is, $R_1$ and $R_2$ correspond to $R_{21}$ and $R_{22}$. However, if the precursor has protecting groups, then $R_1$ and/or $R_2$ - depending on whether both residues have a protecting group or only one of them - can be converted to the residues $R_{21}$ and $R_{22}$ by cleaving-off the protecting groups.

The conversion of the compound of formula V-a to a compound of formula XI-a is shown in scheme 2a, the conversion of the compound of formula V-b to a compound of formula XI-b is shown in scheme 2b:



scheme 2a

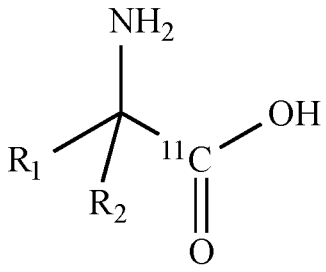



V-b XI-b

scheme 2b

The reaction according to scheme 2a is particularly advantageous if the method according to the invention is to be employed for the preparation of L-amino acids and their derivatives.

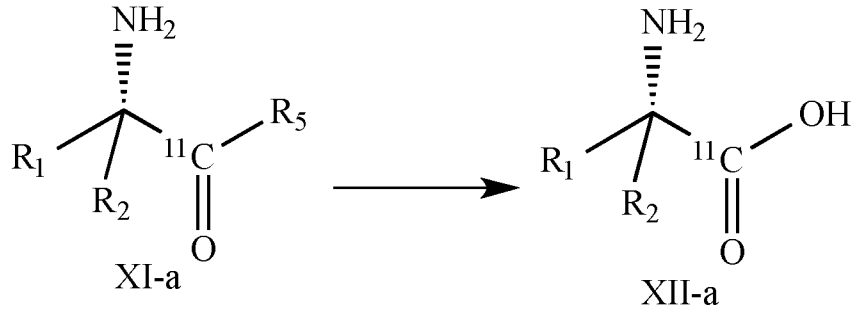
Subsequently, the compound of formula XI can be converted to a compound of formula XII by means of hydrolysis:



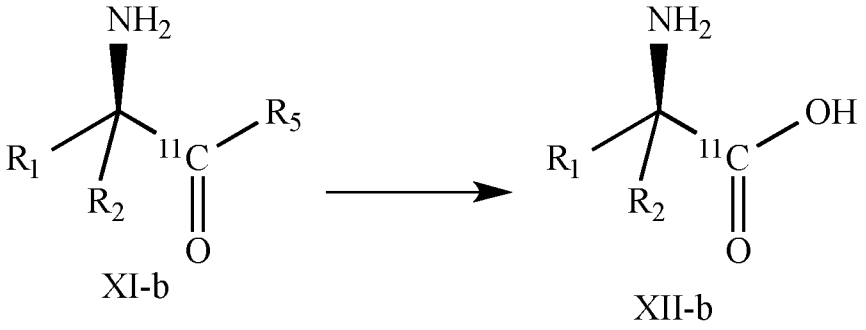

(formula XII)

wherein $R_1$ and $R_2$ are defined above. The compound of formula XII comprises only amino acids. Apart from that and provided that the precursor has no protecting group, the compound of formula XII corresponds to the compound of formula X. That is, $R_1$ and $R_2$ correspond to $R_{21}$ and $R_{22}$. However, if the precursor has protecting groups, then $R_1$ and/or $R_2$ - depending on whether both residues have a protecting group or only one of them - can be converted to the residues $R_{21}$ and $R_{22}$ by cleaving-off the protecting groups. The hydrolysis can be carried out under reaction conditions known to the skilled person. The hydrolysis can be carried out under acidic or basic conditions.

The conversion of the compound of formula XI-a to a compound of formula XII-a by means of hydrolysis is shown in scheme 3a, the conversion of the compound of formula XI-b to a compound of formula XII-b by means of hydrolysis is shown in scheme 3b:




scheme 3a

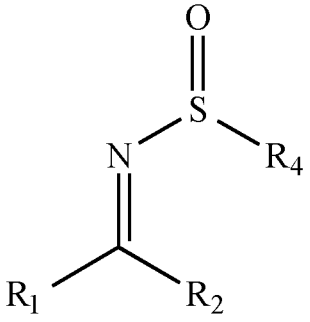



scheme 3b

The reaction according to scheme 3a is particularly advantageous if the method according to the invention is to be employed for the preparation of L-amino acids and their derivatives.

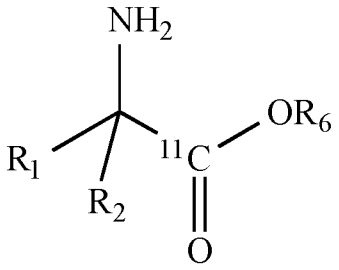
Instead of an alcoholysis of the compound of formula V to a compound of formula XI and the optional subsequent hydrolysis of the compound of formula XI to a compound of formula XII a direct hydrolysis of formula V to a compound of formula XII may be provided.

In a preferred embodiment of the method according to the invention a precursor of formula II



(formula II)

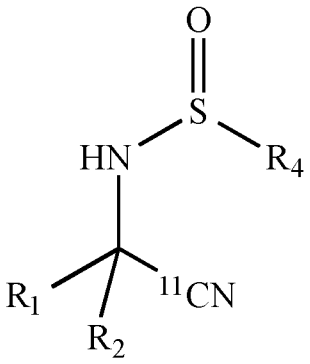
is used for the preparation of a compound of formula XIII:



(formula XIII)

wherein $R_1$, $R_2$, $R_4$, and $R_6$ have the meanings given above. Provided that residues $R_1$ and/or $R_2$ have no protecting groups, compound XIII can correspond to compound X, wherein $R_5$ is -O-$R_6$. $R_4$ is preferably tert.-butyl, $R_2$ preferably hydrogen. If residues $R_1$ and/or $R_2$ have protecting groups, then the compound of formula XIII can be converted to a compound of formula X by cleaving-off the protecting groups.

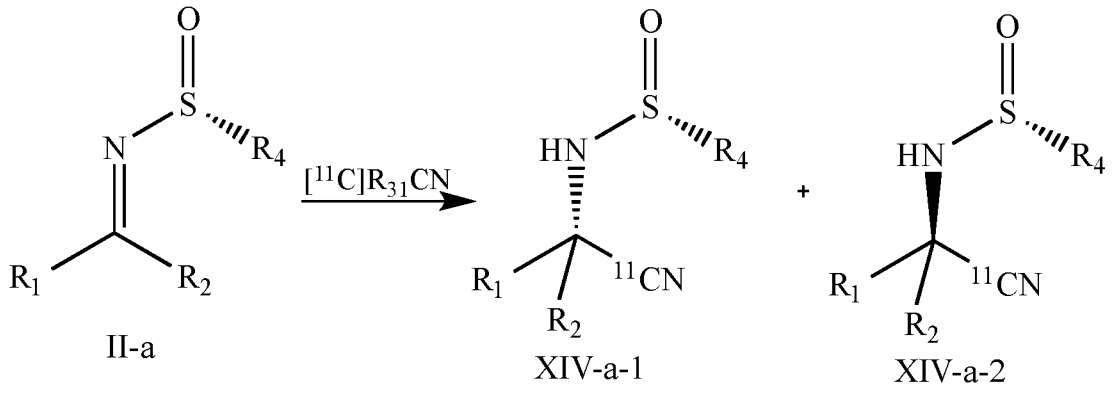
First, the preferred embodiment can include the reaction of the compound of formula II with the carbon-11 labelled synthon to a compound of formula XIV:



(formula XIV)

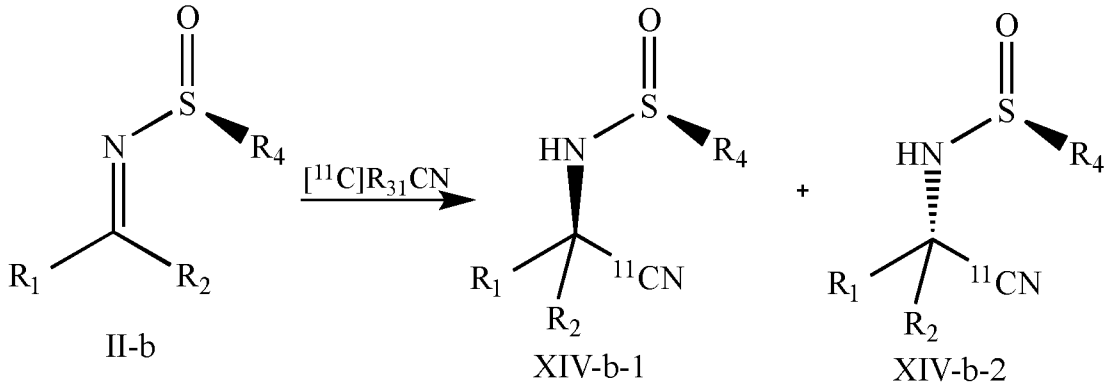
wherein $R_1$, $R_2$, and $R_4$ have the meanings given above. The reaction conditions are preferably selected such that the carbon-11 labelled synthon binds to the precursor by nucleophilic addition. Preferably, the reaction takes place in an aprotic solvent, for example 1,4-dioxane, tetrahydrofurane, diethylether, methyl-tert-butylether, toluene, benzol, dichloromethane, and other halogenated solvents, wherein the listing is not complete, a protic organic solvent, for example methanol, ethanol, iso-propanol, n-propanol, n-butanol, or the solvents water, DMSO, and DMF, wherein the listing is not

complete, or mixtures of an aprotic solvent and a protic organic solvent. A solvent mixture is preferred that has a blending ratio of the aprotic solvent and the protic organic solvent in any conceivable ratio. A solvent mixture is preferred that has a blending ratio of the aprotic solvent and the protic organic solvent in any conceivable ratio, preferably in the range of 7 to 3 to 9 to 1 based on the volume. Particularly preferred is a blending ration of 8 to 2 based on the volume. A particularly preferred solvent mixture is a mixture of 1,4-dioxane and methanol. The reaction is preferably carried out at a temperature of 10 to 60°C, particularly preferred at room temperature. The reaction time may be between 1 and 10 min. The reaction mixture in addition to the precursor, the carbon-11 labelled synthon, and the solvent can contain one or more excipients. Examples of such an excipient are cesium fluoride, tetrabutylammonium fluoride, tin diiodide, aluminum trichloride, or other Lewis acids or fluoride salts. When using [$^{11}$C]HCN as the synthon, then the labelling yields are 70 to 95% with respect to the [$^{11}$C]HCN employed. The conversion of the compound of formula II-a to a mixture of the enantiomers of formula XIV-a-1 and XIV-a-2 is shown in scheme 4a, the conversion of the compound of formula II-b to a mixture of the enantiomers of formula XIV-b-1 and XIV-b-2 is shown in scheme 4b. The chiral auxiliary of the precursor and the reaction conditions selected, especially the excipient(s) employed and/or the solvent(s) employed determine which of the two reaction products is obtained in excess.




II-a XIV-a-1 XIV-a-2

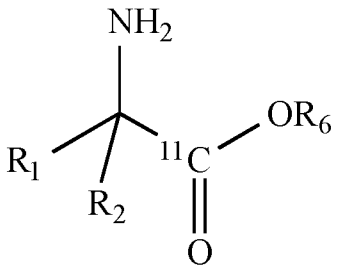
scheme 4a




scheme 4b

The reaction according to scheme 4b is particularly advantageous if the method according to the invention is to be employed for the preparation of L-amino acids and their derivatives.

Subsequently, the compound of formula IV can be converted to a compound of formula XV by means of alcoholysis:

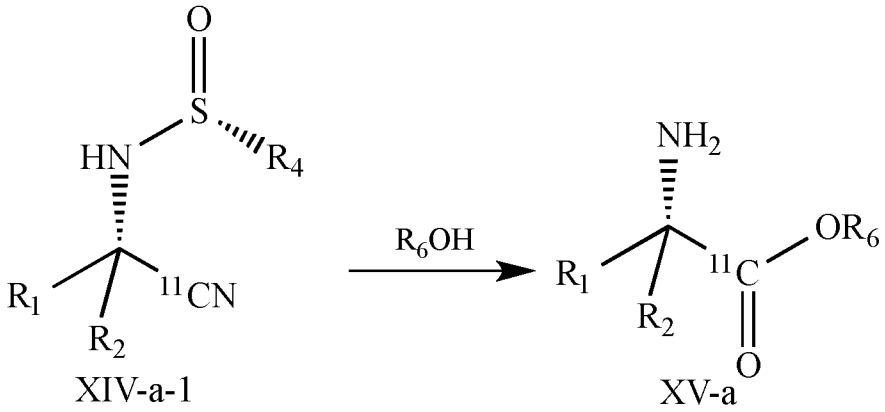



(formula XV)

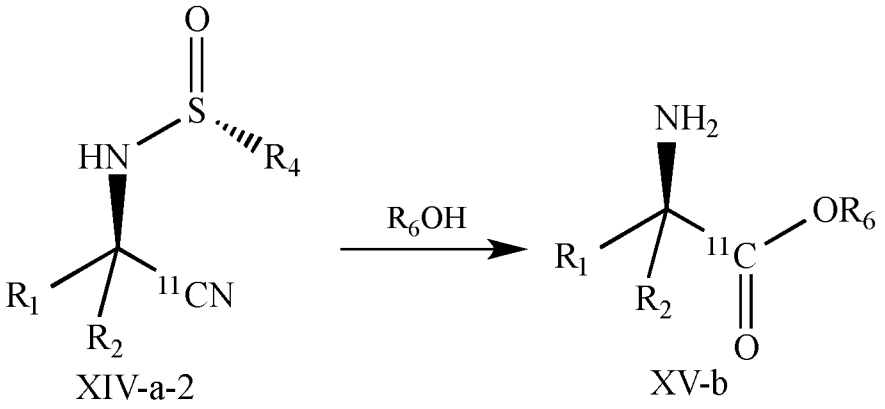
wherein $R_1$, $R_2$, and $R_6$ are as defined above, with the provision that $R_6$ is not hydrogen. The compound of formula XV comprises no amino acids. Apart from that and provided that the precursor has no protecting group, the compound of formula XV corresponds to the compound of formula X. That is, $R_1$ and $R_2$ correspond to $R_{21}$ and $R_{22}$. However, if the precursor has protecting groups, then $R_1$ and/or $R_2$ - depending on whether both residues have a protecting group or only one of them - can be converted to the residues $R_{21}$ and $R_{22}$ by cleaving-off the protecting groups.

The alcoholysis is carried out by employing an alcohol that has the group $R_6$ and is illustrated in schemes 5a and 5b as $R_6OH$. The alcoholysis can be carried out under reaction conditions known to the skilled person. The reaction time may for example be 5

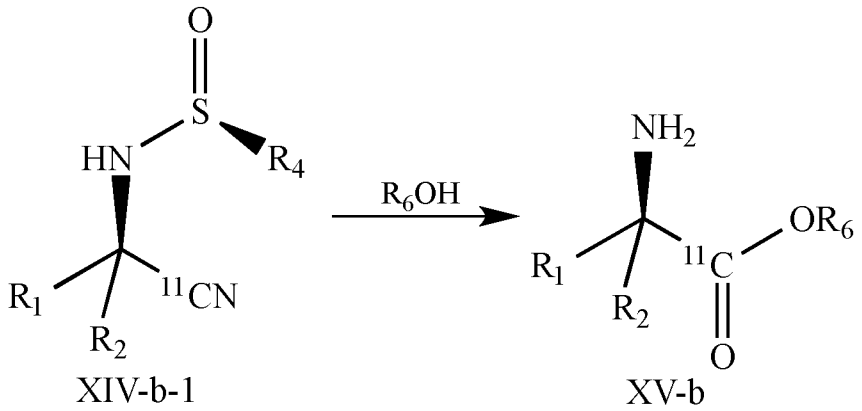
to 60 minutes, the reaction temperature may for example be 60 to 150°C. The conversion of the compound of formula XIV-a-1 to a compound of formula XV-a is shown in scheme 5a-1, the conversion of the compound of formula XIV-a-2 to a compound of formula XV-b is shown in scheme 5a-2, the conversion of the compound of formula XIV-b-1 to a compound of formula XV-b is shown in scheme 5b-1, and the conversion of the compound of formula XIV-b-2 to a compound of formula XV-a is shown in scheme 5b-2.



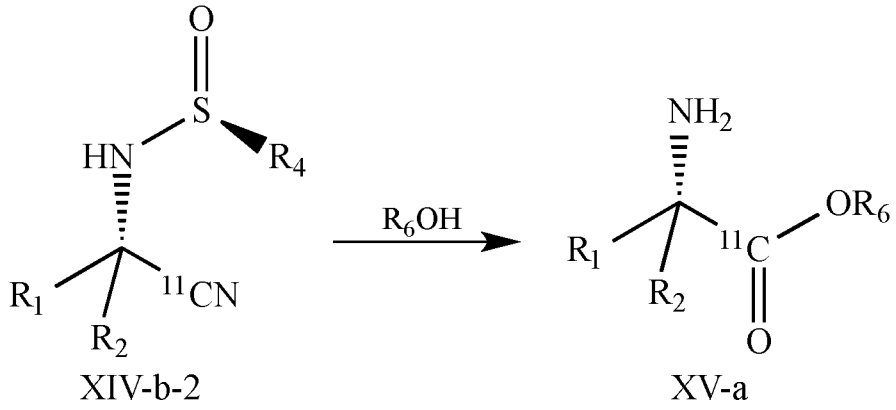

scheme 5a-1




scheme 5a-2



scheme 5b-1

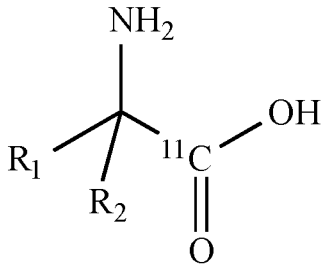



XIV-b-2 XV-a

scheme 5b-2

For the preparation of L-amino acids and their derivatives there is preferably prepared the compound of formula XV-a.

Subsequently, the compound of formula XV can be converted to a compound of formula XII by means of hydrolysis:

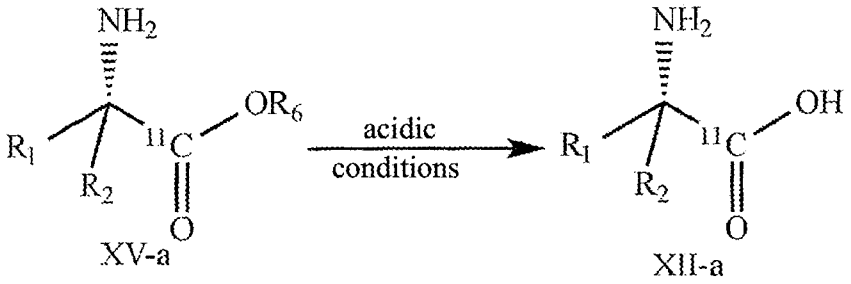



(formula XII)

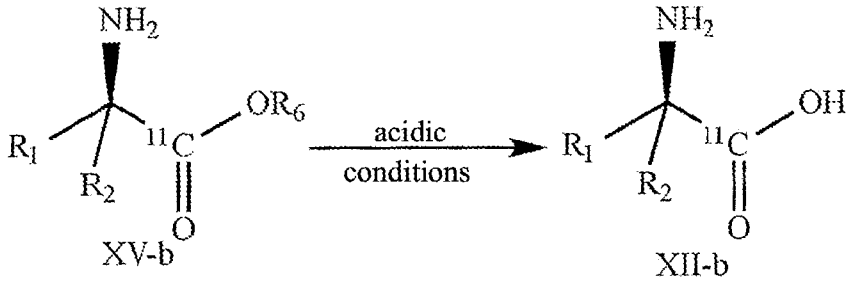
wherein $R_1$ and $R_2$ are as defined above. Provided that the precursor has no protecting group, the compound of formula XII corresponds to the compound of formula X, wherein $R_5$ is hydroxy. That is, $R_1$ and $R_2$ correspond to $R_{21}$ and $R_{22}$. However, if the precursor has protecting groups, then $R_1$ and/or $R_2$ - depending on whether both residues have a protecting group or only one of them - can be converted to the residues $R_{21}$ and $R_{22}$ by cleaving-off the protecting groups.

The hydrolysis can be carried out under acidic conditions. For example, aqueous concentrated hydrochloric acid can be employed for the hydrolysis. The reaction temperature may be for example 100 to 250°C, the reaction time may be 2 to 20 min. The conversion of the compound of formula XV-a to a compound of formula XII-a by

means of hydrolysis is shown in scheme 6a, the conversion of the compound of formula XV-b to a compound of formula XII-b by means of hydrolysis is shown in scheme 6b:



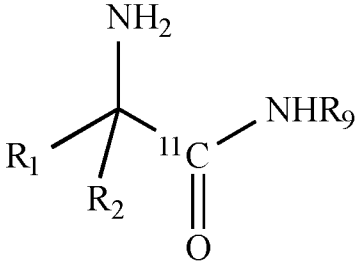

scheme 6a




scheme 6b

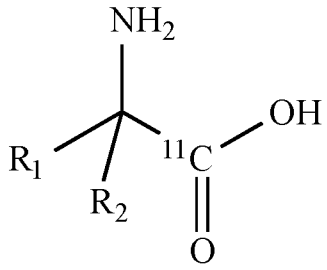
For the preparation of L-amino acids and their derivatives the compound of formula XV-a is reacted to the compound of formula XII-a. For the preparation of D-amino acids and their derivatives the compound of formula XV-b is reacted to the compound of formula XII-b.

As an alternative to an alcoholysis of the compounds of formula XIV which can optionally be followed by a hydrolysis the compounds of formula XIV can directly be subjected to a direct hydrolysis. Here, the compound of formula XIV first is converted to a compound of formula XVI:




(formula XVI)

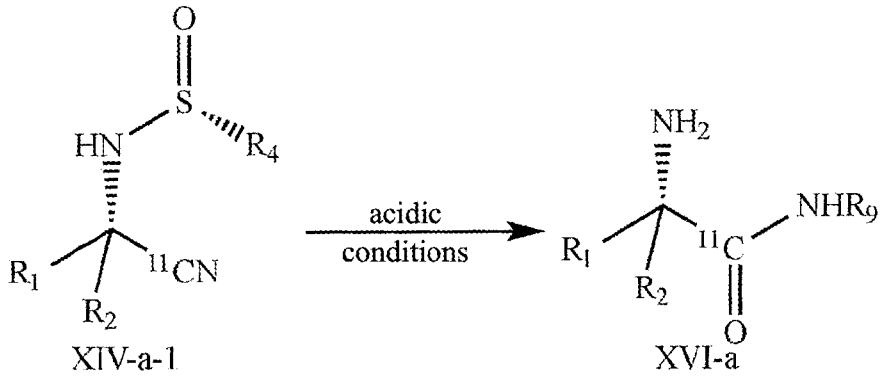
wherein $R_1$ and $R_2$ are defined as in claim 6 and $R_9$ is selected from the group comprising hydrogen, unsubstituted or substituted $C_1$-$C_6$alkyl, unsubstituted or substituted $C_2$-$C_6$alkenyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. Preferably, $R_9$ is hydrogen. Then, the hydrolysis can be continued by converting the compound of formula XVI to a compound of formula XII:



(formula XII)

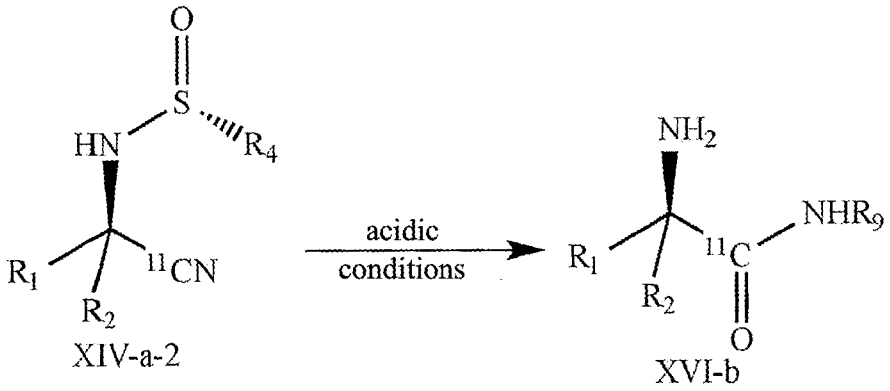
wherein $R_1$ and $R_2$ are as defined above. The direct hydrolysis can be carried out under acidic or basic conditions, preferably acidic conditions. For example, aqueous concentrated hydrochloric acid can be employed for the acidic hydrolysis. The reaction temperature may for example be 100 to 250°C, the reaction time may be 2 to 20 min.

The conversion of the compound of formula XIV-a-1 to a compound of formula XVI-a is shown in scheme 7a-1, the conversion of the compound of formula XIV-a-2 to a compound of formula XVI-b is shown in scheme 7a-2, the conversion of a compound of formula XIV-b-1 to a compound of formula XVI-b is shown in scheme 7b-1, and the conversion of a compound of formula XIV-b-2 to a compound of formula XVI-a is shown in scheme 7b-2:

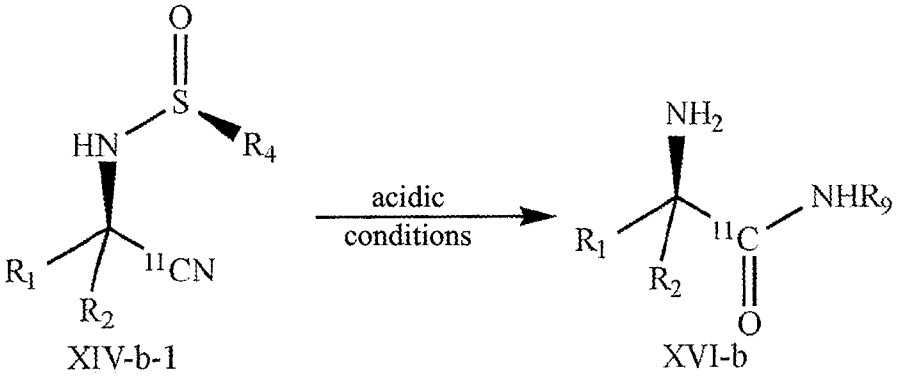



XIV-a-1 XVI-a

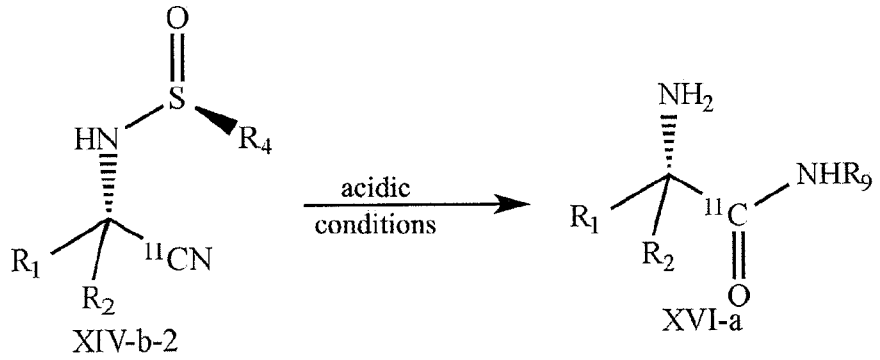
scheme 7a-1




scheme 7a-2

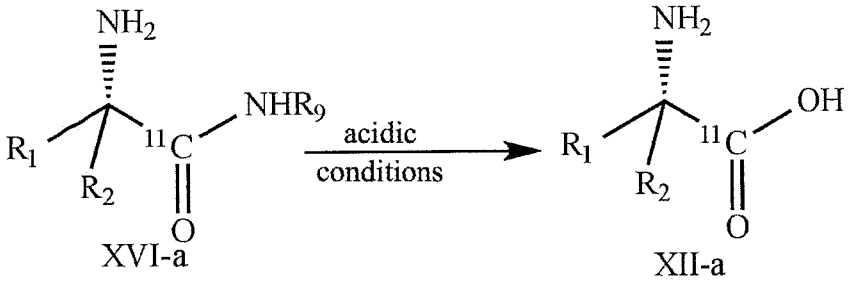



scheme 7b-1

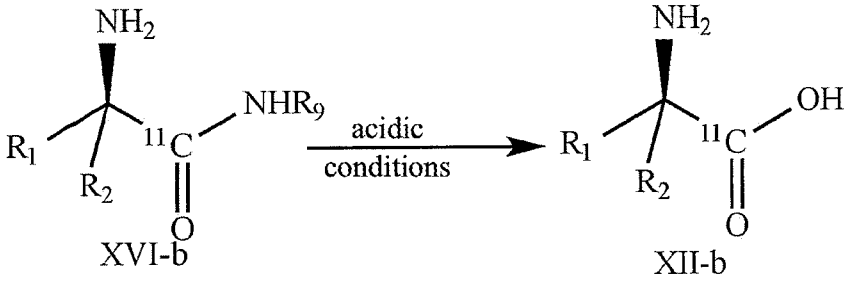



scheme 7b-2

The conversion of the compound of formula XVI-a to a compound of formula XII-a by means of hydrolysis is shown in scheme 8a, the conversion of the compound of formula XVI-b to a compound of formula XII-b by means of hydrolysis is shown in scheme 8b:




scheme 8a




scheme 8b

For the preparation of L-amino acids and their derivatives the compound of formula XVI-a is reacted to the compound of formula XII-a. For the preparation of D-amino acids and their derivatives the compound of formula XVI-b is reacted to the compound of formal XII-b.

If residues $R_1$ and $R_2$ are unprotected residues, then it may be provided that residues $R_1$ and $R_2$ of a compound of formulae XI, XII, XIII, XV, or XVI correspond to residues $R_{21}$ and/or $R_{22}$ of the compound X. However, if residue $R_1$ is a protected residue, then it may be provided that residue $R_1$ of a compound of formulae XI, XII, XIII, XV, or XVI is converted to residue $R_{21}$ of compound X by cleaving-off one or more protecting groups. If residue $R_2$ is a protected residue, then it may be provided that residue $R_2$ of a compound of formulae XI, XII, XIII, XV, or XVI is converted to residue $R_{22}$ of the compound X by cleaving-off one or more protecting groups.

The method according to the invention can be carried out in a microfluidics system, especially a microfluidics reactor. The microfluidics system may for example be the ISAR® platform offered by General Electric. The microfluidics system may include cleaning cartridges to subject the target compound, i.e. a compound of formula X, or an intermediate to cleaning. The microfluidics system may have a polymer chip on which

the synthesis of the carbon-11 labelled amino acids or derivatives thereof can be carried out. The method according to the invention may be an automated method.

The method according to the invention is preferably carried out as a non-carrier associated synthesis (n. c. a. synthesis).

To obtain an enantiomerically pure compound of formula X the method according to the invention can include a purification step to separate the undesired enantiomer. The purification step may for example be an enzymatic purification or a chiral separation. If it is the aim of the method according to the invention to prepare an L-amino acid or a derivative thereof, then the corresponding D-amino acid or its derivative, respectively can be separated by means of the purification step.

The method according to the invention permits synthesis times of less than 20 min for the preparation of carbon-11 labelled amino acids or their derivatives, in fact including (i) the conversion of $[^{11}C]CO_2$ to $[^{11}C]R_{31}CN$, (ii) the incorporation of $[^{11}C]R_{31}CN$ into the precursor, and (iii) the alcoholysis, and optionally subsequent hydrolysis, or the direct hydrolysis of the nitrile group to the optionally substituted carboxylic acid.

The carbon-11 labelled amino acids prepared by means of the method according to the invention or derivatives thereof may further by reacted to peptides, especially oligopeptides, or peptide analogues. The carbon-11 labelled amino acids prepared by means of the method according to the invention or derivatives thereof may be used for the incorporation into peptides, proteins, or antibodies.

Without wishing to be bound to an explanation the invention is based on the following findings: By targeted selection of the optimum solvent, preferably 1,4-dioxane in a ratio to methanol of 4:1, and of the excipients, preferably cesium fluoride (CsF) high diastereomeric excesses (de) in very short reaction times can be achieved. On the one hand in pure dioxane, dichloromethane, toluene, or other aprotic non-polar solvents by adding cesium fluoride in fact the de values are very good (70% to >90%). But CsF is only hardly soluble in these solvents which leads to the fact that the reaction time is unexpectedly extended to several hours or days, respectively. On the other hand, the excipient CsF in fact is better soluble in protic polar solvents such as water, DMSO, or various alcohols such as methanol, ethanol, and propanol. Thus, the reaction times

decrease to less than one hour. However, stereoselectivity changes for the worse (<50%) and even turns around in that occasion. These two opposite effects have led to the realization that in principle by suitably selecting the solvent(s) and/or the excipient(s) both the L-amino acid and the D-amino acid can be isolated from the same enantiomerically pure (S)-sulfinyl imine precursor with good stereoselectivity (>30% ee or de, respectively in case of the intermediate compound). The inventors have found that in a mixture of dioxane with little methanol or water, i.e. a proportion preferably of 10 to 20% based on the volume, solubility of the excipient CsF increases strongly and thus, the reaction time decreases to less than 20 min. Here, the de values were at good 70 to 80%. After the release of the enriched amino acid or its derivative by acidic hydrolysis, with a basic hydrolysis being also possible, an enzymatic purification may take place. For example, the D-amino acid can be removed by immobilized D-α-amino acid oxidase (D-AAO). This is also possible vice versa. By the use of the enzyme L-α-amino acid oxidase (L-AAO) in principle it is also possible to isolate the enantiomeric D-amino acid.

By means of the methods according to the invention amino acids and derivatives thereof can be obtained – without purification such as an enzymatic or chiral purification – with an *ee* value of 30% or more, preferably 50% or more, more preferably 70% or more, and particularly preferred 90% or more. By means of the methods according to the invention specific activities of ca. 20 to 70 GBq/µmol can be obtained. Higher specific activities can be achieved which i.a. requires the employment of extremely pure gases, especially nitrogen and/or hydrogen.

The advantages of the methods according to the invention over the method by Xing et al. particularly lie in the diastereoselective reaction of a chiral precursor with $[^{11}C]R_{31}CN$. It is possible to specifically prepare L or D amino acids. Moreover, the precursor needs not to be intermediately prepared in the synthesis module, but is thermodynamically stable and thus, can be stored.

According to the invention there may further be provided a reagent kit that can include a precursor according to the invention for the enantio-selective preparation of a carbon-11 labelled amino acid or a derivative thereof, a solvent and one or more excipients. Additionally, the reagent kit according to the invention can include one or more cleaning cartridges. The reagent kit according to the invention permits the automated enantio-selective preparation of the carbon-11 labelled amino acid or of the derivative thereof.

The invention is explained in detail with respect to examples not intended to limit the invention. Here,

Fig. 1 shows a radio-chemical HPLC chromatogram showing the results of the labelling reaction in the diastereo-selective preparation of [1-$^{11}$C]-L tyrosine (compound X-12);

Fig. 2 shows a radio-chemical HPLC chromatogram showing the reaction products after the hydrolysis and prior to the purification;

Fig. 3 shows a radio-chemical HPLC chromatogram showing the results of the purification with an SPE cartridge; and

Fig. 4 shows a radio-chemical HPLC chromatogram showing the results of the enzymatic purification.

General synthesis rule for the diastereo-selective labelling of □-amino acids with [$^{11}$C]HCN

Start: Formation of [$^{11}$C]HCN from [$^{11}$C]$CO_2$

First, the [$^{11}$C]$CO_2$ formed in the cyclotron is directed into the hot cell and absorbed there on a molecular sieve (4Å). In the meantime, nitrogen flows through the molecular sieve and entrains undesired impurities. Now, the molecular sieve is heated to a temperature of 350°C, wherein from ca. 200°C the captured [$^{11}$C]$CO_2$ is desorbed from the molecular sieve and is passed on via the nitrogen flow (40 mL/min). In the next reaction step hydrogen gas (20 mL/min) is passed to the nitrogen/[$^{11}$C]$CO_2$ mixture and said gas mixture is passed over a nickel catalyst of 400°C. In this process the [$^{11}$C]$CO_2$ is reduced to $^{11}$C methane ([$^{11}$C]$CH_4$) and water. Water and unreacted [$^{11}$C]$CO_2$ are captured (with Ascarite and Sicapent) and gaseous ammonia (5 mL/min) is added to the remaining methane flow. Said gas mixture is passed over a platinum wire at 950°C and thereby [$^{11}$C]HCN is formed. Now, said gas flow is directly used for the synthesis of amino acids.

A) Labelling Reaction

A precursor of formula II, preferably of formula III (between 0.5 and 5 mg) and cesium fluoride (1-2 eq), dissolved in ca. 200-800 µL of a solution mixture consisting of 1,4-

dioxane and methanol (blending ratio 7:3 to 9:1, preferably 8:2 based on the volume) are added into a small reactor vial (V=1-3 mL). The radio-labelled, gaseous [$^{11}$C]HCN is introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 50-500 mL/min. The reaction is carried out at room temperature, but also higher and lower temperatures are possible, e.g. 10 to 60°C.

Depending on the adjusted gas flow the complete formation of [$^{11}$C]HCN takes between 1 and 10 minutes. After completion of the [$^{11}$C]HCN formation reaction hydrogen gas (ca. 20-150 mL/min) is briefly (0.1-5 min) passed through the reactor vial to remove excessive ammonia. In this way, the amino acid nitrile of formula II, preferably of formula V is formed as an intermediate compound. Typically, the labelling rates are between 70-95% of the [$^{11}$C]HCN employed.

B) Automated Hydrolysis

After completed labelling reaction the reaction solution is transferred to a further reactor vial (V=2 to 4 mL). (However, this is not required. Rather, the hydrolysis can also be carried out in the same reactor vial). The reactor vial is heated from the outside and hydrogen gas is passed through the reaction solution, whereby the solution is somewhat concentrated. Next, concentrated HCl (400-1000 µL) is added to said reactor vial and the reaction solution is heated for 2-20 min at 100-250°C. After completed hydrolysis sufficient buffer ($K_3PO_4$) is added to the solution so that the pH value of the solution is adjusted to pH=2-2.5. Advantageously, the pH value is ca. 2 points below the isoelectric point of the target compound, i.e. an α-amino acid of formula X.

C) Purification

Purification of the target compound obtained in step B) can be done automatically or manually. For the manual purification the solution is applied to a conditioned SPE cartridge (SPE = solid phase extraction) (e.g., PSH+ or SCX by Machery Nagel®, similar cartridges are available from other suppliers) such that the target compound is bound and the by-product can be separated. The SPE cartridge is purged with so-called washing solutions ($H_2O$, acetonitrile (ACN), diluted acids such as acetic acid (AcOH)) and the purified free target compound is eluted with a suitable eluting agent (e.g., 0.5M aqueous NaOH or 0.4M $NH_3$ in $H_2O$/ACN) (with other eluting agent solutions being also possible).

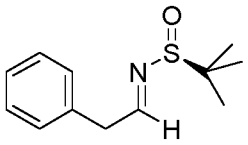
A buffer mixture (tris-base, FAD solution) is added to the eluate such that the pH value of the solution is ca. 8-9 and the tris-concentration is 50 mM, the FAD concentration is 10 µM (with other buffer mixtures being possible, FAD = flavine-adenine dinucleotide, however which is not mandatory). Said solution is slowly applied over a cartridge filled with 0.1-2 g of immobilized D-α-amino acid oxidase (D-AAO).

There is obtained a compound of formula X with $R_5$ being hydroxy with ca. 30 to 50% decomposition corrected yield (10 to 15% not decomposition corrected, based on [$^{11}$C]HCN formed, with higher yields being also possible), with >95% radio-chemical purity and an ee value of 100%. Without enzymatic purification the amino acid is obtained with an ee value of 70 to 90%.

Example 1: Synthesis of L-[1-$^{11}$C]phenylalanine (Compound X-11)

A) Labelling with carbon-11

The phenylalanine-sulfinylimine precursor (*S*,*E*)-2-methyl-*N*-(2-phenylethylidene)-propane-2-sulfinamide (formula III-a-11, 2.1 mg)




(formula III-a-11)

and cesium fluoride (2.1 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the [1-$^{11}$C]phenylalanine-nitrile as an intermediate compound with labelling rates of 73±3% of the [$^{11}$C]HCN employed.

B) Hydrolysis

Next, concentrated HCl (600 µl) was added to said reactor vial and the reaction solution was heated for 5 min at 150°C. After completed hydrolysis the solution is added to a 10 mL vial in which 2.5 mL of an aqueous 1M $K_3PO_4$ solution have been present, so that the pH value of the solution was adjusted to pH=2-2.5.

C) Purification

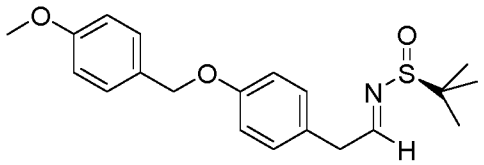
The solution was applied to a conditioned SPE cartridge (e.g., PSH+ by Machery Nagel), 4 mL/min. The SPE cartridge was successively purged with 2 mL of 3% acetic acid (AcOH), 2 mL of acetonitrile (ACN), and 5 mL of $H_2O$ and the [1-$^{11}$C]phenylalanine was eluted with 1.5 mL of 0.5M aqueous NaOH. 500 µL of a tris-buffer (500 mM tris-base, 50 µM of flavine-adenine dinucleotide, pH 7.5) were added to the eluate and the solution was slowly applied to a cartridge which was filled with 0.5 g of immobilized D-alpha amino acid oxidase (D-AAO).

There was obtained L-[1–$^{11}$C]phenylalanine in ca. 30±3% time-corrected yield (based on [$^{11}$C]HCN formed), with a >95% radio-chemical purity and an *ee* value of 100%.

Example 2: Synthesis of L-[1-$^{11}$C]tyrosine (Compound X-12)

A) Labelling with carbon-11

The tyrosine-sulfinylimine precursor (*S*,*E*)-*N*-(2-(4-(4-methoxybenzyloxy)phenyl)-ethylidene)-2-methylpropane-2-sulfinamide (formula III-a-12, protecting group p-methoxybenzyl, 2.7 mg)



(formula III-a-12)

and cesium fluoride (1.7 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the para-methoxybenzyl-protected [1–$^{11}$C]tyrosine-nitrile as an intermediate compound with labelling rates of 88±5% of the [$^{11}$C]HCN employed.

B) Hydrolysis and cleaving-off of the protecting group

Now, the reactor vial was heated from the outside and hydrogen gas was passed through the reaction solution, whereby the solution was somewhat concentrated. Next, concentrated HCl (800 µL) was added to said reactor vial and the reaction solution was heated for 5 min at 150°C. After completed hydrolysis 3 mL of an aqueous 1M $K_3PO_4$

solution were added to the solution, so that the pH value of the solution was adjusted to pH=2-2.5.

C) Purification

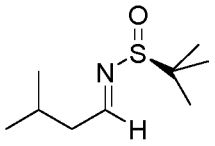
Purification was carried out as in example 1. There was obtained L-[1–$^{11}$C]tyrosine in ca. 39±6% time-corrected yield (based on [$^{11}$C]HCN formed), with >95% radio-chemical purity and an *ee* value of 100%.

Figures 1 to 4 show radio-chemical HPLC chromatograms which confirm the enantio-selective preparation of the target compound X-12. Fig. 1 shows the results of the labelling reaction. At 13.207 min there is found the (S)-N-((R)-1-cyano-2-(4-(4-methoxybenzyloxy)phenyl)ethyl)-2-methylpropane-2-sulfinamide, at 13.684 min there is found the (S)-N-((S)-1-cyano-2-(4-(4-methoxybenzyloxy)phenyl)ethyl)-2-methyl-propane-2-sulfinamide. This confirms that one of the two diastereomers is particularly preferably formed. Fig. 2 shows the reaction products after hydrolysis and prior to purification. It can be seen that the target compound is present in a high excess over the other enantiomer (D-tyrosine). Fig. 3 shows the results of the purification with an SPE cartridge and Fig. 4 shows the results of the enzymatic purification. It can be seen that by the purification steps the enantiomeric purity can be further increased.

Example 3: Synthesis of L-[1-$^{11}$C]leucine (Compound X-13)

A) Labelling with carbon-11

The leucine-sulfinylimine precursor (*S*,*E*)-2-methyl-*N*-(3-methylbutylidene)propane-2-sulfinamide (formula III-a-13, 2.0 mg)




(formula III-a-13)

and cesium fluoride (2.4 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the [1–$^{11}$C]leucine-nitrile as an intermediate compound with labelling rates of 70±10% of the [$^{11}$C]HCN employed.

B) Hydrolysis

Hydrolysis was carried out as in example 1, except that the hydrolysis temperature was 130°C.

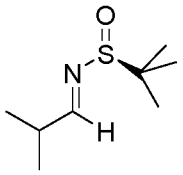
C) Purification

Purification was carried out as in example 1. There was obtained L-[1–$^{11}$C]leucine in ca. 35±5% time-corrected yield (based on the [$^{11}$C]HCN formed) with >95% radio-chemical purity and an *ee* value of 100%.

Example 4: Synthesis of L-[1-$^{11}$C]valine (compound X-14)

A) Labelling with carbon-11

The valine-sulfinylimine precursor (*S*,*E*)-2-methyl-*N*-(2-methylpropylidene)propane-2-sulfinamide (formula III-a-14 1.8 mg)



(formula III-a-14)

and cesium fluoride (2.3 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the [1–$^{11}$C]valine-nitrile as an intermediate compound with labelling rates of 70±3% of the [$^{11}$C]HCN employed.

B) Hydrolysis

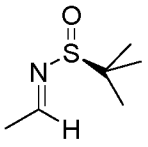
Hydrolysis was carried out as in example 1.

C) Purification

Purification was carried out as in example 1. There was obtained L-[1–$^{11}$C]valine in ca. 18±6% time-corrected yield (based on the [$^{11}$C]HCN formed) with >95% radio-chemical purity and an *ee* value of 100%.

Example 5: Synthesis of L-[1-$^{11}$C]alanine (compound X-15)

The alanine-sulfinylimine precursor (*S*,*E*)-*N*-ethylidene-2-methylpropane-2-sulfinamide (formula III-a-15 1.8 mg)

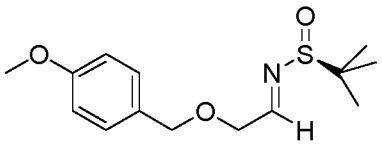


(formula III-a-15)

and cesium fluoride (2.8 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the [1–$^{11}$C]alanine-nitrile as an intermediate compound with labelling rates of 76±5% of the [$^{11}$C]HCN employed.

Example 6: Synthesis of L-[1-$^{11}$C]serine (compound X-16)

The serine-sulfinylimine precursor (S,E)-N-(2-(4-methoxybenzyloxy)ethylidene)-2-methylpropane-2-sulfinamide (Compound III-a-16, protecting group p-methoxybenzyl, 2.4 mg)

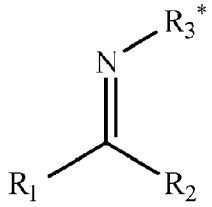


(formula III-a-16)

and cesium fluoride (1.9 mg, 1.5 eq), dissolved in 450 µL of a solvent mixture consisting of 1,4-dioxane and methanol (blending ratio 8:2 based on the volume) were added to a small reactor vial (V=1.3 mL). The radio-labelled, gaseous [$^{11}$C]HCN was introduced into said reactor vial with a carrier gas flow (mix of $N_2$, $H_2$, and $NH_3$) of ca. 65-70 mL/min from below for 8 min at room temperature. There was formed the para-methoxybenzyl-protected [1–$^{11}$C]serine-nitrile as an intermediate compound with labelling rates of 78±5% of the [$^{11}$C]HCN employed.

## PATENTKRAV

1. Anvendelse af en precursor til fremstilling af carbon-11-mærkede aminosyrer eller derivater deraf, hvor precursoren er en forbindelse med formlen I:




(Formel I)

hvor

$R_1$ og $R_2$ uafhængigt er valgt fra gruppen omfattende hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, der eventuelt kan modificeres ved at indsætte mindst én gruppe X i carbonkæden, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, der eventuelt kan modificeres ved at indsætte mindst én gruppe X i carbonkæden, substitueret eller usubstitueret alkylaryl, substitueret eller usubstitueret arylalkyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl,

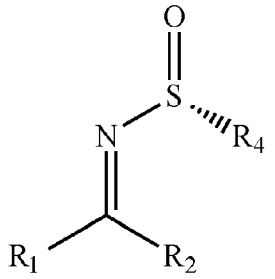
$R_3$ er et chiralt hjælpestof valgt fra gruppen omfattende substitueret eller usubstitueret $C_1$-$C_6$-alkylsulfinyl, substitueret eller usubstitueret arylsulfinyl, substitueret eller usubstitueret arylalkyl og substitueret eller usubstitueret arylglycinol,

X er valgt fra gruppen omfattende oxygen, svovl, -SO-, -$SO_2$- og -N($R_{10}$)-,

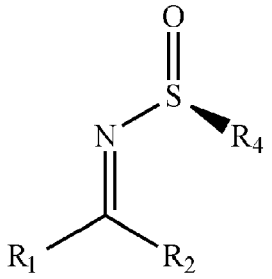
$R_{10}$ omfatter hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl; og

resterne eventuelt er ubeskyttede eller beskyttede.

2. Anvendelse ifølge krav 1, kendetegnet ved, at precursoren er en forbindelse med formlen II-a eller II-b:



(Formel II-a)



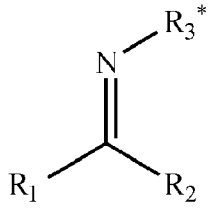
(Formel II-b)

hvor $R_1$ og $R_2$ er som defineret i krav 1, og $R_4$ er valgt fra gruppen omfattende usubstitueret eller substitueret $C_1$-$C_6$-alkyl og substitueret eller usubstitueret aryl.

3. Anvendelse ifølge krav 2, kendetegnet ved, at $R_1$ er valgt fra gruppen omfattende usubstitueret eller substitueret $C_1$-$C_6$-alkyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl; $R_2$ er hydrogen; og $R_4$ er som defineret i krav 2.

4. Anvendelse ifølge et hvilket som helst af de foregående krav, kendetegnet ved, at $R_1$ er valgt fra gruppen omfattende methyl, beskyttet eller ubeskyttet hydroxymethyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butanyl, sek-butyl, tert-butyl, phenyl, beskyttet eller ubeskyttet hydroxyphenyl, benzyl og beskyttet eller ubeskyttet hydroxybenzyl, og $R_2$ er hydrogen.

5. Fremgangsmåde til diastereo-selektiv mærkning af en precursor med formel I:



(Formel I)

hvor

$R_1$ og $R_2$ uafhængigt er valgt fra gruppen omfattende hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, der eventuelt kan modificeres ved at indsætte mindst én gruppe X i carbonkæden, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, der eventuelt kan modificeres ved at indsætte mindst én gruppe X i carbonkæden, substitueret eller usubstitueret alkylaryl, substitueret eller usubstitueret

arylalkyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl,

$R_3$ er et chiralt hjælpestof valgt fra gruppen omfattende substitueret eller usubstitueret $C_1$-$C_6$-alkylsulfinyl, substitueret eller usubstitueret arylsulfinyl, substitueret eller usubstitueret arylalkyl og substitueret eller usubstitueret arylglycinol,

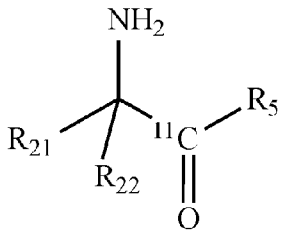
X er valgt fra gruppen omfattende oxygen, svovl, -SO-, -$SO_2$- og -N($R_{10}$)-,

$R_{10}$ omfatter hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl; og

resterne eventuelt er ubeskyttede eller beskyttede,

kendetegnet ved, at precursoren omsættes med en carbon-11-mærket synthon til en carbon-11-mærket forbindelse, som har en carbon-11-mærket carbonylgruppe.

6. Fremgangsmåde ifølge krav 5, kendetegnet ved, at den carbon-11-mærkede forbindelse er en forbindelse med formlen X:




(Formel X)

hvor

$R_{21}$ og $R_{22}$ har de i krav 1 til 4 angivne betydninger for $R_1$ og $R_2$,

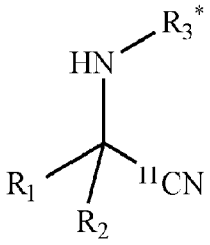
$R_5$ er valgt fra gruppen bestående af $OR_6$ og $NR_7R_8$,

$R_6$, $R_7$ og $R_8$ uafhængigt er valgt fra gruppen omfattende hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl,

hvor en precursor ifølge et hvilket som helst af kravene 1 til 4 omsættes med en carbon-11-mærket synthon til forbindelsen med formlen X.

7. Fremgangsmåde ifølge krav 5 eller krav 6, kendetegnet ved, at den carbon-11-mærkede synthon er [$^{11}$C]$R_{31}$CN, hvor $R_{31}$ er valgt fra gruppen omfattende hydrogen, et alkalimetal såsom lithium, natrium eller kalium, acetyl og alkylsilyl.

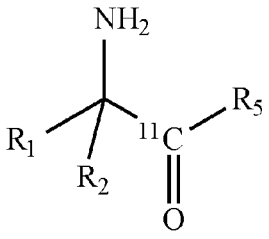
8. Fremgangsmåde ifølge et hvilket som helst af kravene 5 til 7, kendetegnet ved, at precursoren omsættes med den carbon-11-mærkede synthon til en forbindelse med formlen V:




(Formel V)

hvor $R_1$, $R_2$ og $R_3$ har de i krav 5 angivne betydninger.

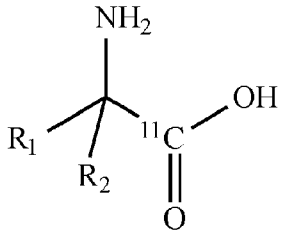
9. Fremgangsmåde ifølge krav 8, kendetegnet ved, at forbindelsen med formlen V omdannes til en forbindelse med formlen XI ved hjælp af alkoholyse eller hydrolyse




(Formel XI)

hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger, og $R_5$ er som defineret i krav 6 med det forbehold, at $R_5$ er ikke OH.

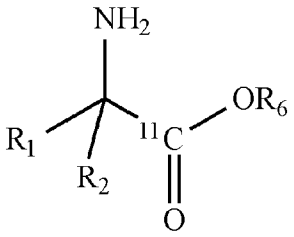
10. Fremgangsmåde ifølge krav 9, kendetegnet ved, at forbindelsen med formlen XI omdannes til en forbindelse med formlen XII ved hjælp af hydrolyse



(Formel XII)

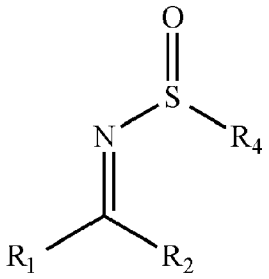
hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger.

11. Fremgangsmåde ifølge et hvilket som helst af kravene 6 til 8, kendetegnet ved, at der til fremstilling af en forbindelse med formlen XIII:



(Formel XIII)

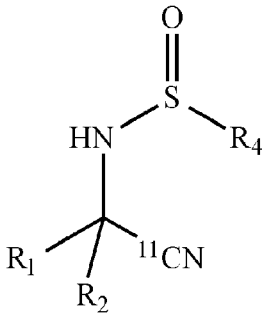
anvendes en precursor med formel II



(Formel II)

hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger, $R_6$ har de i krav 6 angivne betydninger, og $R_4$ har den i krav 2 angivne betydning.

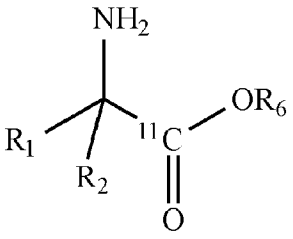
12. Fremgangsmåde ifølge krav 11, kendetegnet ved, at en forbindelse med formlen II omsættes med den carbon-11-mærkede synthon til en forbindelse med formlen XIV:



(Formel XIV)

hvor $R_1$ og $R_2$ har de i krav 6 angivne betydninger, og $R_4$ har den i krav 2 angivne betydning.

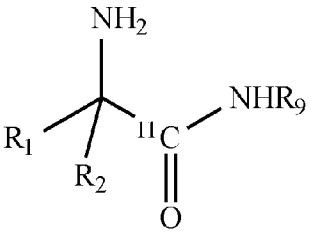
13. Fremgangsmåde ifølge krav 12, kendetegnet ved, at forbindelsen med formlen XIV omdannes til en forbindelse med formlen XV ved hjælp af alkoholyse:



(Formel XV)

hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger, og $R_6$ er som defineret i krav 6 med det forbehold, at $R_6$ ikke er hydrogen.

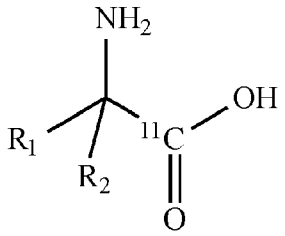
14. Fremgangsmåde ifølge krav 12, kendetegnet ved, at forbindelsen med formlen XIV omdannes til en forbindelse med formlen XVI ved hjælp af hydrolyse:



(Formel XVI)

hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger, og $R_9$ er valgt fra gruppen omfattende hydrogen, usubstitueret eller substitueret $C_1$-$C_6$-alkyl, usubstitueret eller substitueret $C_2$-$C_6$-alkenyl, substitueret eller usubstitueret aryl og substitueret eller usubstitueret heteroaryl.

15. Fremgangsmåde ifølge krav 13 eller krav 14, kendetegnet ved, at forbindelsen med formlen XV eller XVI omdannes til en forbindelse med formlen XII ved hjælp af hydrolyse:




(Formel XII)

hvor $R_1$ og $R_2$ har de i krav 1 til 4 angivne betydninger.

16. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, kendetegnet ved, at resterne $R_1$ og $R_2$ i en forbindelse med formlerne XI, XII, XIII, XV eller XVI svarer til resterne $R_{21}$ og/eller $R_{22}$ i forbindelse X, forudsat at resterne $R_1$ og $R_2$ er ubeskyttede, eller at resten $R_1$ i en forbindelse med formlerne XI, XII, XIII, XV eller XVI omdannes til resten $R_{21}$ i forbindelse X ved at fraspalte én eller flere beskyttelsesgrupper, og/eller resten $R_2$ i en forbindelse med formlerne XI, XII, XIII, XV eller XVI omdannes til resten $R_{22}$ i forbindelse X ved at fraspalte én eller flere beskyttelsesgrupper.

17. Kit til enantio-selektiv fremstilling af en carbon-11-mærket aminosyre eller et derivat deraf med en precursor ifølge et hvilket som helst af kravene 1 til 4, ét eller flere opløsningsmidler, én eller flere excipienser og én eller flere rensepatroner.

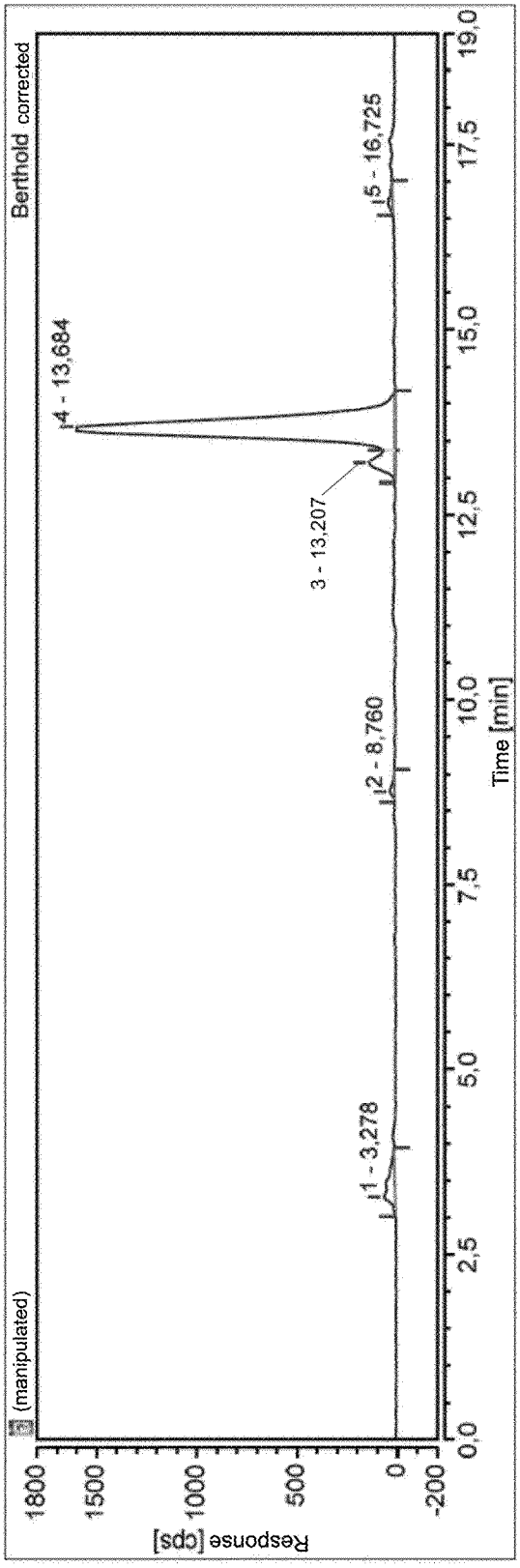

Berthold corrected
(manipulated)
Response [cps]
1800
1500
1000
500
0
-200
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
19,0
Time [min]
1 - 3,278
2 - 8,760
3 - 13,207
4 - 13,684
5 - 16,725

Fig. 1

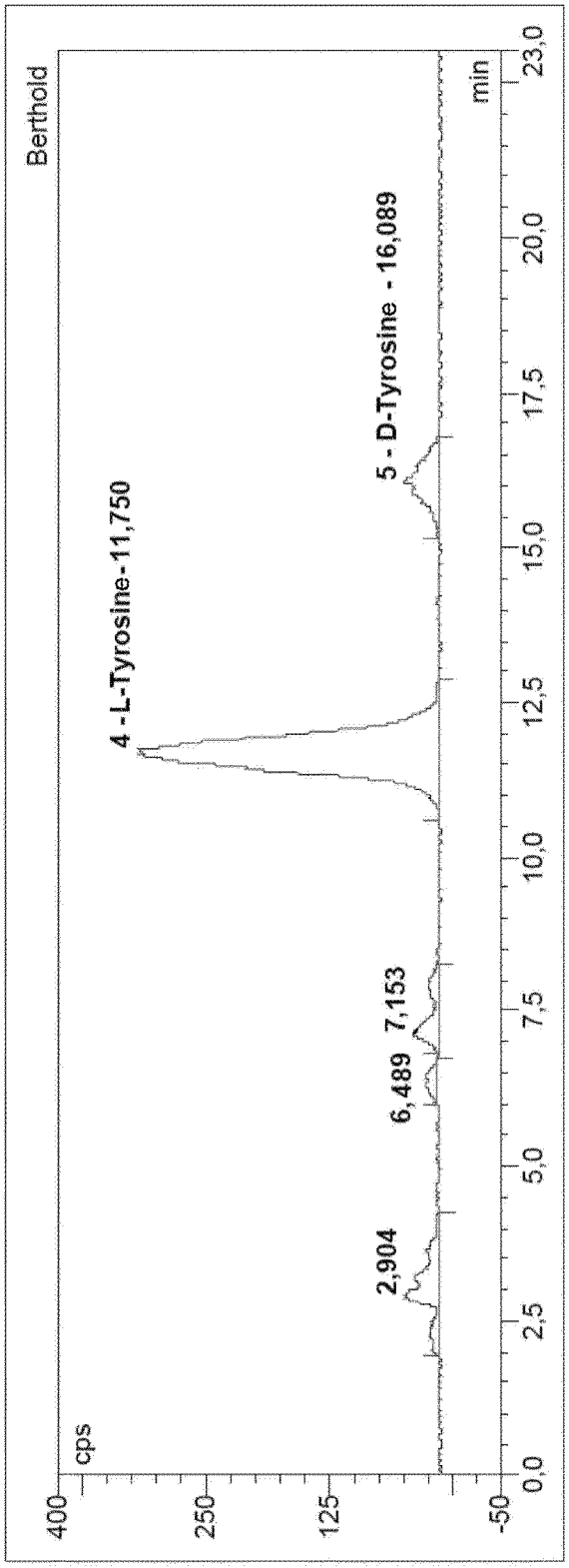

Berthold
cps
400
250
125
-50
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
20,0
23,0
min
4 - L-Tyrosine - 11,750
5 - D-Tyrosine - 16,089
2,904
6,489
7,153

Fig. 2

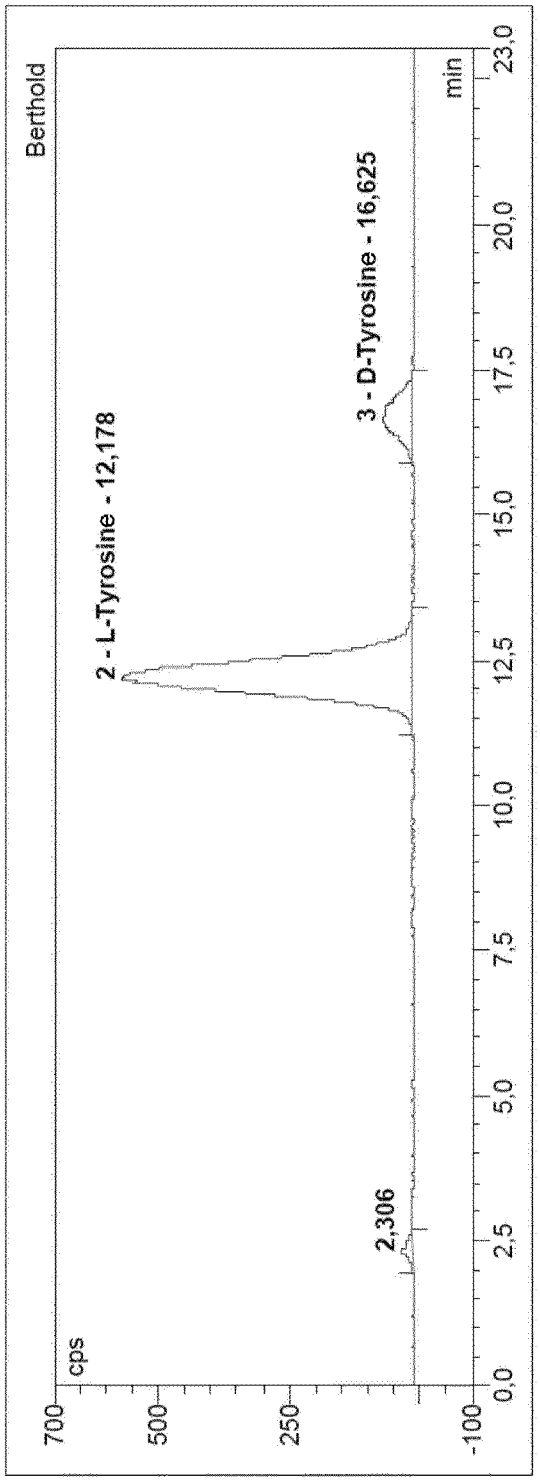

Berthold
cps
700
500
250
-100
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
20,0
23,0
min
2,306
2 - L-Tyrosine - 12,178
3 - D-Tyrosine - 16,625

Fig. 3

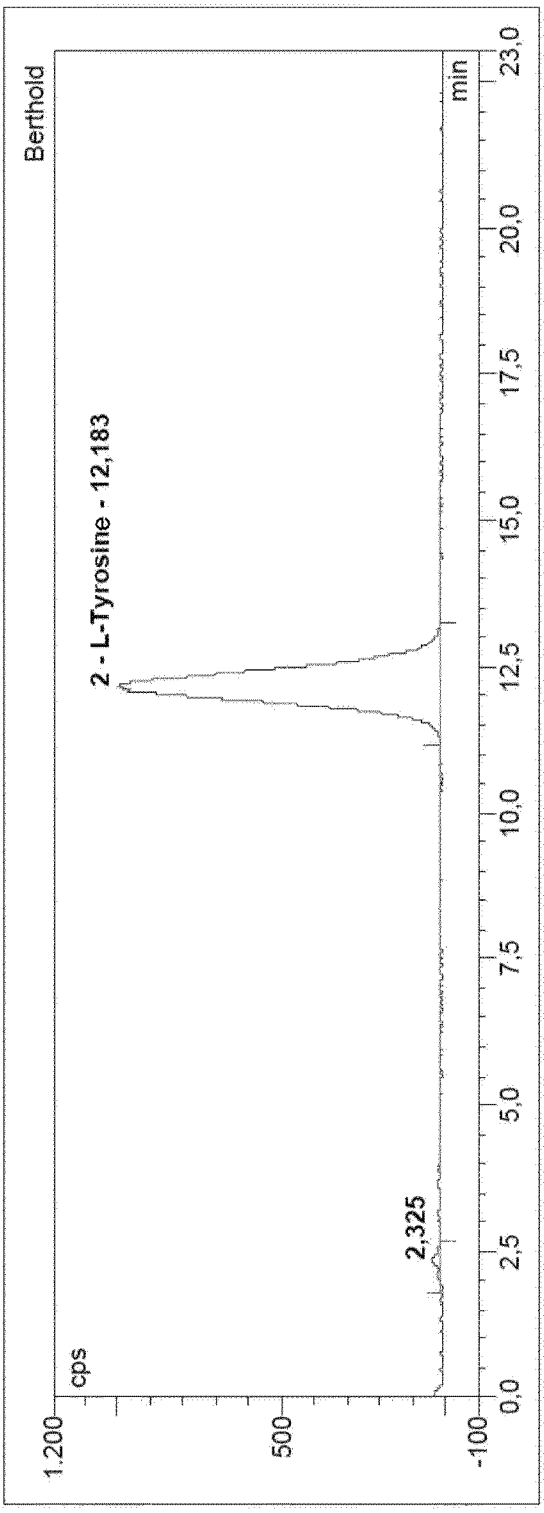

Berthold
cps
1.200
500
-100
0,0
2,5
5,0
7,5
10,0
12,5
15,0
17,5
20,0
23,0
min
2 - L-Tyrosine - 12,183
2,325

Fig. 4