

METHOD FOR PREPARING METHIONINE ANALOGUES

The present invention concerns a method for preparing methionine analogues, as well as selenium derivatives of methionine analogues, from abundant and accessible compounds, derived from biomass.

The methionine and the analogues thereof such as 2-hydroxy-4-methylthiobutanoic acid (**HMTBA**) and 2-oxo-4-methylthiobutanoic acid (**KMB**), as well as salts, chelates, in particular metal chelates (of Zn, Ca Mn, Mg, Cu, Na...) and the esters of these acids, such as the isopropyl and tert-butyl esters of HMTBA, are widely used in animal nutrition. The selenium derivatives of the methionine and of the hydroxy-analogues thereof are also constituents of major interests in animal nutrition.

Given the continuously growing volumes, consumed worldwide, of these ingredients, it is necessary to develop manufacturing methods from renewable, energy-efficient and non-polluting sources.

The authors have thus developed a method for preparing these compounds from organic acids, the salts or the derivatives thereof, which may be obtained from biomass, in particular by biological transformations such as fermentation methods.

According to the invention, this method allows preparing a compound or a salt thereof, said compound having the formula (I),



Where X is selected from O; N-R' where R' represents H or a C1-C6 alkyl group; and N-OR'' where R'' represents H, a C1-C6 alkyl group or an alkylaryl group;

R¹ represents H or a C1-C6 alkyl group;

R² represents H, a C1-C6 alkyl group, or an alkylaryl group; and

R³ represents CH₂SR⁴ or CH₂SeR⁴ with R⁴ representing H or a C1-C6 alkyl group,

from a compound of formula (II), or a salt thereof,



Where R¹, R² and X have the definition above; and

R^5 represents H or $COOR^6$ with R^6 representing H or a C1-C6 alkyl group,

This method allows manufacturing methionine analogues such as KMB, from acids such as the oxaloacetic acid and the pyruvic acid, in interesting yields for an industrial exploitation, not releasing sub-products in excessive amounts and involving moderate reaction conditions and available reagents. Moreover, these compounds constitute particularly interesting precursors of methionine in its different active forms D,L; D and L and of HMTBA in its different enantiomeric forms D,L; D and L. They may be indeed transformed into said methionine or said HMTBA by simple reduction, for example with hydrides of $NaBH_4$ type, or by catalytic or biocatalytic hydrogenation, or by racemic or enantioselective biochemical transformation, or by any other method known to those skilled in the art.

More specifically, the method of the invention is carried out in the presence of a compound of formula (III)



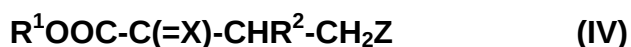
Where Y represents H; OR^7 with R^7 representing H, a C1-C6 alkyl group or an acyl group of formula $CO-R^4$ with R^4 meeting the preceding definition; SR^4 or SeR^4 with R^4 meeting the preceding definition; or NR^8R^9 , with R^8 and R^9 , identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group;

Z, identical to or different from Y, represents OR^{10} with R^{10} representing H, a C1-C6 alkyl group, or $CO-R^4$ with R^4 meeting the preceding definition; a cyclic or acyclic $N(COR^4)(COR^4)$ group, with R^4 meeting the preceding definition; or a $NR^{11}R^{12}$ group, with R^{11} and R^{12} , identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group;

Or Y and Z represent together $=O$;

And said method comprises the following steps:

The compound (II) is reacted with the compound (III) to lead to an intermediate having the structure (IV)



Where R^1 , R^2 , X and Z have the definition above,

The compound (IV) thus obtained is reacted with R^4SH or a salt thereof, or R^4SeH or a salt thereof, with R^4 meeting the preceding definition, already present in the reaction medium or added during the method,

Then, upon completion of the reaction, the compound (I) or a salt thereof is isolated.

As will be described hereinafter, according to a variant of the method, the reaction of the compound (IV) with R^4SH or a salt thereof, or R^4SeH or a salt thereof, with R^4 meeting the preceding definition, already present in the reaction medium or added during the method, leads to a compound having the structure (V)



Where R^1 , R^2 and Z have the definition above

A represents OH ; $HN-R'$ where R' represents H or a C1-C6 alkyl group; or $HN-OR''$ where R'' represents H , a C1-C6 alkyl group, or an alkylaryl group; and

B represents SR^4 or SeR^4 with R^4 meeting the preceding definition.

Before exposing the invention in more details, some terms used in the text are specified below.

In the formulas defining the compounds and reagents obtained or involved, the term « alkyl » designates a linear or branched monovalent hydrocarbon radical having 1 to 20 carbon atoms, advantageously 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, neopentyl, *n*-hexyl or a cyclic monovalent hydrocarbon radical having 3 to 20 carbon atoms, advantageously 3 to 6 carbon atoms, such as cyclopropyl, cyclohexyl.

Alkylaryl group means an aryl group comprising 6 to 20 carbon atoms, said aryl group being substituted by at least one alkyl group meeting the definition above.

The invention is detailed hereinafter and its advantageous variants are presented.

The method disclosed above can be implemented according to several approaches.

A first route preferably consists in reacting a compound (II) with the formaldehyde or the paraformaldehyde, in hydrated or non-hydrated form, in a

basic medium and in the presence of MeSH or a salt thereof, such as sodium, potassium or calcium salts of methylmercaptan.

A second route includes reacting a compound (II) with a compound (III), said compound (III) being selected from 1-[(methylsulfanyl)methyl]-piperidine, 1-[(methylsulfanyl)methyl]-pyrrolidine and 1-[(methylsulfanyl)methyl]-diethylamine. This second route leads to an intermediate compound which may be isolated or not, which is an object of the present invention. This compound meets the following formula (V):



Where R^1 represents H or a C1-C6 alkyl group;

R^2 represents H, a C1-C6 alkyl group, or an alkylaryl group;

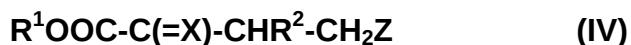
A represents OH, $\text{HN-R}'$ where R' represents H or a C1-C6 alkyl group, or $\text{HN-OR}''$ where R'' represents H or a C1-C6 alkyl group or an alkylaryl group;

B represents SR^4 or SeR^4 with R^4 representing H or a C1-C6 alkyl group;

Z represents OR^{10} with R^{10} representing H; a C1-C6 alkyl group; a CO- R^4 group with R^4 representing H or a C1-C6 alkyl group, a cyclic or acyclic $\text{N(COR}^4\text{)(COR}^4\text{)}$ group, with R^4 meeting the preceding definition; or $\text{NR}^{11}\text{R}^{12}$, with R^{11} and R^{12} , identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group.

The second route advantageously brings into contact the oxaloacetic acid or an ester thereof, that is to say a compound of formula (II) in which X represents O, R^2 represents H and R^5 represents CO_2R^6 with R^6 representing H or a C1-C6 alkyl group, with a compound of formula (III) of the type $\text{CH}_2(\text{Y})(\text{Z})$ in which Y and Z represent respectively the group SCH_3 and the group $\text{NR}^{11}\text{R}^{12}$ as defined previously; preferably, the group $\text{NR}^{11}\text{R}^{12}$ represents the piperidinyl group. Therefore, the invention also concerns the compound of formula (V) in which A represents OH, B represents SCH_3 , R^2 represents H and Z represents the piperidinyl group.

According to a third route, it is possible to react the compound (II) with a compound (III) selected from methylenedipiperidine, methylenedipyrrolidine and methylenedi(diethylamine). An intermediate compound of this third route, which can be isolated or not, is also an object of the invention. It meets the following formula (IV):



Where X is selected from O; N-R' where R' represents H or a C1-C6 alkyl group; and N-OR'' where R'' represents H, a C1-C6 alkyl group or an aryl group;

R¹ represents H or a C1-C6 alkyl group;

R² represents H, a C1-C6 alkyl group, or an alkylaryl group; and

Z represents NR⁸R⁹, with R⁸ and R⁹, identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group;

As will be illustrated in the examples, the third route advantageously brings into contact the oxaloacetic acid or an ester thereof, that is to say a compound of formula (II) in which X represents O, R² represents H and R⁵ represents CO₂R⁶ with R⁶=H, with a compound of formula (III) in which Y and Z represent respectively the group NR⁸R⁹ and the group NR¹¹R¹² as previously defined. Preferably, at least one of NR⁸R⁹ and NR¹¹R¹², but even better both, represent the same piperidinyl group. Thus, the invention concerns the intermediate compound of formula (IV) in which X represents O, R² represents H and Z represents the piperidinyl group, R¹ being as previously defined, namely H when the compound (II) is the oxaloacetic acid or a C1-C6 alkyl group when the compound (II) is the corresponding ester of the oxaloacetic acid.

Regardless of the retained route, the compound (II) is advantageously selected from oxaloacetic acid and pyruvic acid.

As previously said, the method of the invention allows obtaining different methionine analogues. Particularly, 2-oxo-4-methylthiobutanoic acid (KMB) or a salt thereof, such as the calcium, magnesium, manganese, copper, zinc, sodium or ammonium salts thereof and the selenium analogue thereof or a salt thereof are products of the method of the invention under economically attractive conditions and yields.

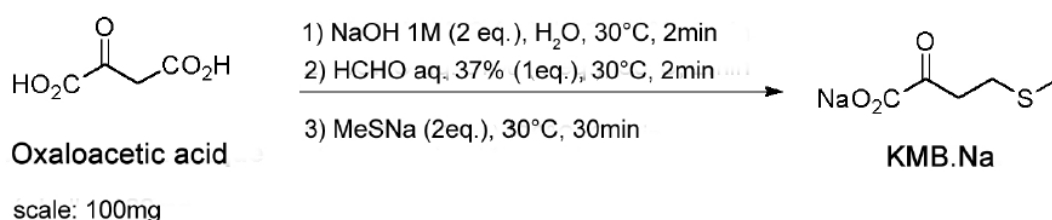
Another object of the invention is a method of D,L-methionine, D- or L-methionine, D,L-2-hydroxy-4-methylthiobutanoic acid (HMTBA), or D- or L-HMTBA, from 2-oxo-4-methylthiobutanoic acid (KMB), said method comprising the preparation of KMB according to the method of the invention as defined above then the transformation of KMB thus obtained into methionine or HMTBA, chemically or biologically, by techniques known to those skilled in the art.

The present invention is described in more details by the following examples illustrating the synthesis of KMB from the oxaloacetic acid and the

pyruvic acid according to different synthetic routes, all falling within the scope of the invention.

Example 1: Preparation of KMB by the first route in the presence of NaOH, HCHO and MeSNa

The general scheme of the synthesis is the following:



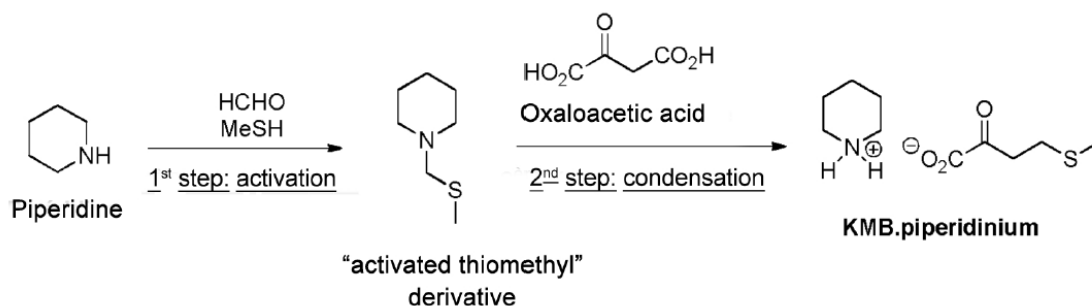
In a reactor, 100 mg of oxaloacetic acid are placed and a solution of NaOH 1M is added (2eq.). The reactor is placed at 30°C and the dissolution of the oxaloacetic acid is immediate. After 2 minutes, the 37%w/w formaldehyde solution is added (1eq). The stirring is maintained for 2 minutes at 30°C then the MeSNa (2eq., 108 mg) is added in one portion and the reaction medium is stirred at 30°C.

A monitoring of the reaction by HPLC-UV (column C18 Hydro-RP) is performed after 10 minutes of contact then every 20 minutes. The best performances were measured after 30 minutes of contact at 30°C with the following results:

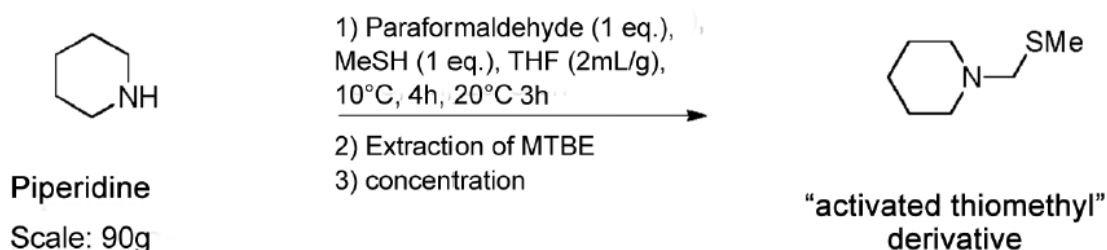
- Conversion_{oxaloacetic acid} = 100%
- Dosed yield in KMB = 75%
- Selectivity in KMB = 75%

Example 2: Preparation of KMB by the second route by using the activated thiomethyl derivative and the oxaloacetic acid

The general scheme of the synthesis is the following:



1st step: Synthesis of the activated thiomethyl derivative [compound (III)]



5

In a 1L reactor under argon, are introduced successively under stirring at 20°C:

- 90.0 g of piperidine
- 180ml of THF
- 34.4g of paraformaldehyde

10

The reaction medium is cooled to 10°C then MeSH is added by bubbling into the reaction medium, at 10°C, until the required amount (1eq.). The addition is completed in 4 hours then the set temperature is raised to 20°C. The reaction medium is stirred for 3h at this temperature. A GC-FID control (column Equity-1) indicates that the conversion of piperidine is complete and the RR_{dosed} in "activated thiomethyl" species is of 97%.

15

180 ml of methyl tert-butyl ether (MTBE) then 180 ml of a NaCl saturated aqueous solution are added to the reaction medium, the two phases are stirred for 5 min and then separated. The organic phase is washed twice with 180 ml of NaCl saturated aqueous solution then dried over Na_2SO_4 and concentrated under reduced pressure (10mbar, 30°C). The "activated thiomethyl" derivative is obtained in the form of a colorless liquid without additional purification (150.4 g). The following performances were obtained:

20

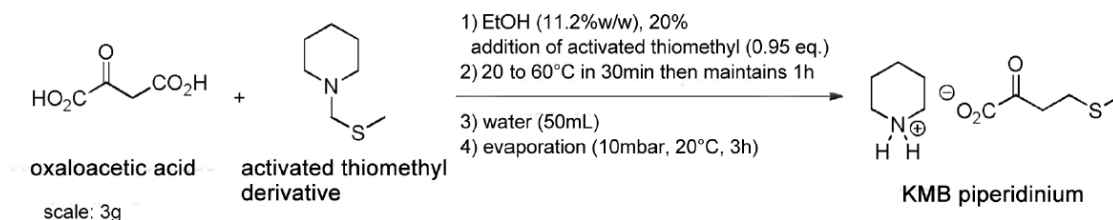
25

- Complete conversion of piperidine

- Isolated yield of activated thiomethyl derivative = 95%
- Titer = 94% (dosed by NMR ^1H vs 3,5-dimethylanisole)

2nd step: Synthesis of KMB piperidinium

5



In a 100mL reactor under argon, provided with a thermostatically-controlled bath, 3g (1 eq.) of oxaloacetic acid and 30ml of ethanol are introduced. After dissolution at 20°C (about 1 minute), the activated thiomethyl derivative is added (1 eq.) then, the medium is heated up to 60°C in 30 minutes then the temperature is maintained for 1 h.

A monitoring of the reaction by HPLC-UV (column C18 Hydro-RP) is performed after 10 minutes of contact then every 20 minutes. The best performances were measured after 1 hour of contact at 60°C with:

- Complete conversion of oxaloacetic acid
- Dosed yield in KMB = 78%
- Selectivity in KMB = 78%

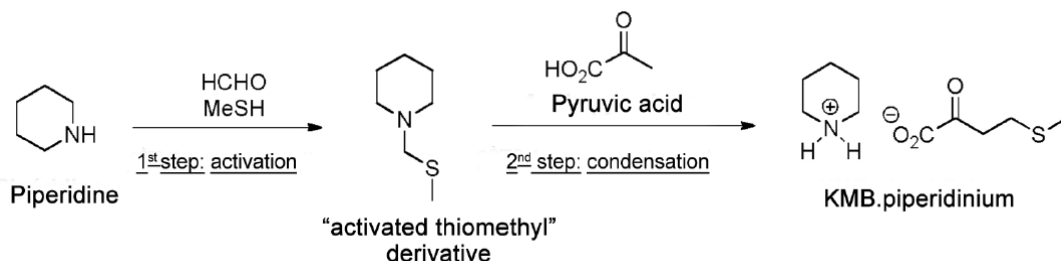
The reaction medium is withdrawn then concentrated under reduced pressure (10mbar, 20°C, 6h). The KMB piperidinium is obtained in the form of a yellow oil without additional purification.

- Isolated yield in KMB piperidinium = 60%
- Titer = 60% (dosed by HPLC vs standard)

Example 3: Preparation of KMB by the second route by using the activated thiomethyl derivative and the pyruvic acid

The general scheme of the synthesis is the following:

25

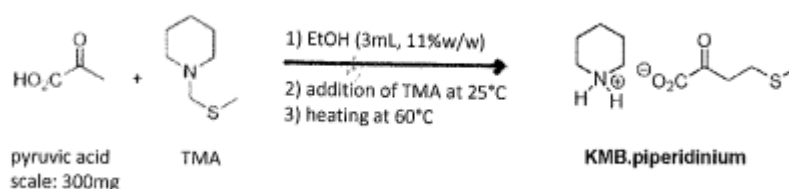


1st step: Synthesis of the activated thiomethyl derivative

It is described in the first step of Example 2.

5

2nd step: Condensation with the pyruvic acid



10 In a Vial, 300 mg (1eq.) of pyruvic acid and 3mL of ethanol are introduced. After dissolution at 20°C, the activated thiomethyl derivative (TMA) is added (1 eq.) then the vial is placed in a plate previously heated to 60°C. The reaction medium is stirred at this temperature for 1 hour.

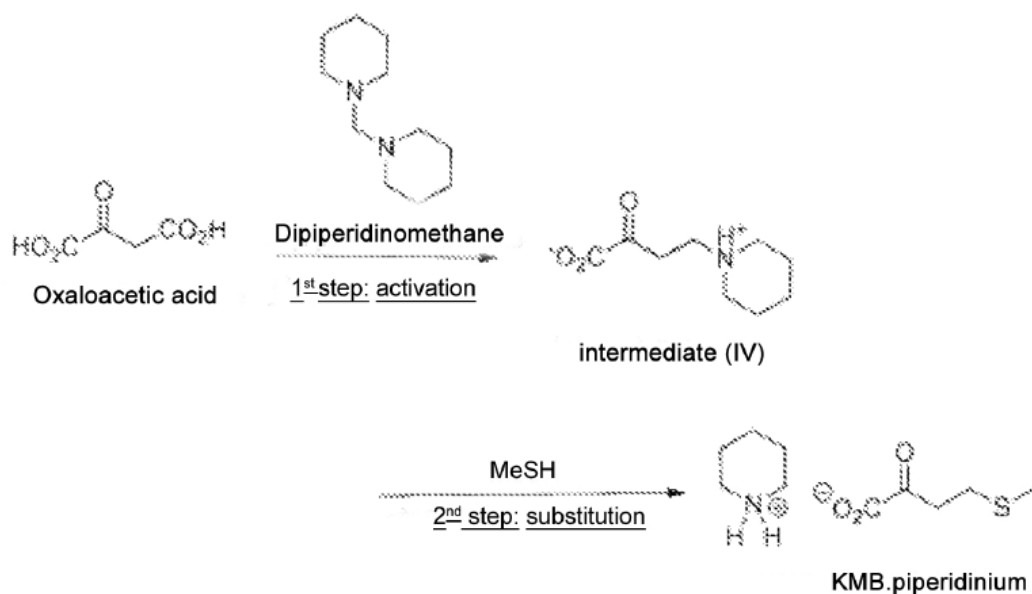
15 A monitoring of the reaction by HPLC-UV (column C18 Hydro-RP) is performed after 15 minutes of contact then every 15 minutes. The best performances were measured after 15min of contact at 60°C with:

- Conversion of the pyruvic acid = 57%
- Yield dosed in KMB = 42%
- Selectivity in KMB = 74%

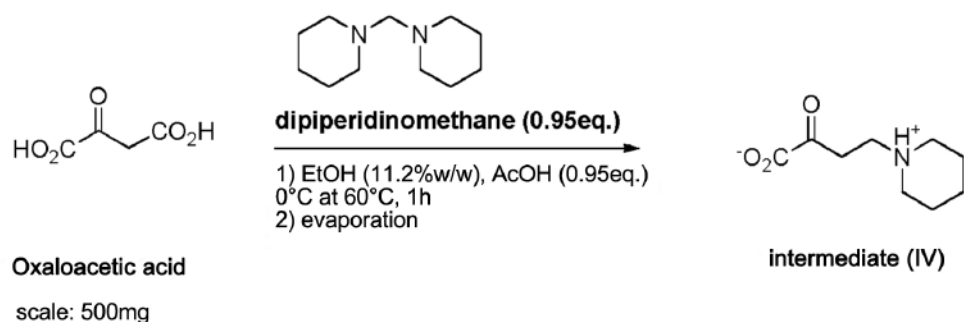
20

Example 4: Preparation of KMB by the third route in a sequenced manner via methylenedipiperidine species

The general scheme of the synthesis is as follows:



1st step: Synthesis of the intermediate (IV)



5

In a 10mL reactor under argon equipped with a temperature probe, are introduced successively at 0°C.

- 500 mg of oxaloacetic acid
- 5mL of EtOH
- 209mg of acetic acid

10

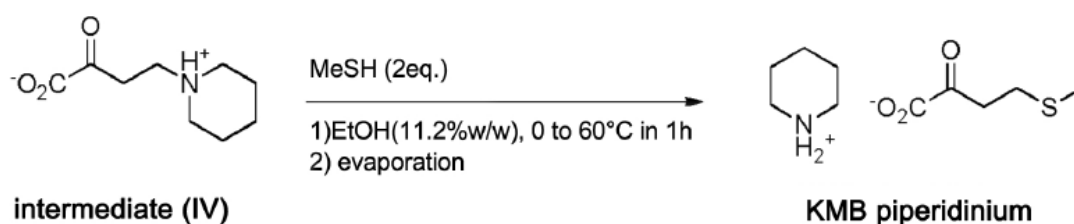
15

After dissolution of the acid (about 5 minutes of stirring), dipiperidinomethane (0.95eq.) is added in 50 minutes via a syringe pump. After the end of the addition, the medium is heated to 60°C in 1 hour then stirred at this temperature for 10 minutes. A control by HPLC-MS (ESI⁺) confirms the formation of the intermediate **IV**.

The reaction medium is cooled to 20°C then the solvent is evaporated under reduced pressure (10mbar, 20°C, 1h) to lead to (IV) in the form of a pale-yellow oil (1.2g).

- Complete TT_{oxaloacetic acid}
- RR_{isolated} of the activated thiomethyl derivative = 60%
- Titer = 35% (dosed by NMR ¹H vs 3,5-dimethylanisole)

2nd step: synthesis of KMB piperidinium



scale: 300mg

In a 10mL reactor under argon provided with a temperature probe, are introduced successively under stirring at 0°C:

- 300mg of intermediate (IV)
- 5mL of EtOH

Once the reaction medium at a temperature lower than 5°C, the MeSH gas is introduced in 1 hour (via a syringe pump). The reaction medium is then heated to 60°C in 1 hour then this temperature is maintained for 30 minutes.

An HPLC control indicates a complete transformation of the intermediate and the majority formation of KMB piperidinium.

The reaction medium is withdrawn then concentrated under reduced pressure (10mbar, 20°C, 1h). The KMB piperidinium is obtained in the form of a yellow oil without additional purification (260 mg)

- Complete TT_{intermediate IV}
- RR_{isolated} of the KMB piperidinium = 90%
- Titer = 45% (dosed by HPLC vs standard)

CLAIMS

1. A method for preparing a compound or a salt thereof, said compound having the formula (I),



Where X is selected from O; N-R' where R' represents H or a C1-C6 alkyl group; and N-OR'' where R'' represents H, a C1-C6 alkyl group or an alkylaryl group;

R¹ represents H or a C1-C6 alkyl group;

R² represents H, a C1-C6 alkyl group, or an alkylaryl group; and

R³ represents CH₂SR⁴ or CH₂SeR⁴ with R⁴ representing H or a C1-C6 alkyl group

from a compound of formula (II), or a salt thereof,



Where R¹, R² and X have the above definition; and

R⁵ represents H or COOR⁶ with R⁶ representing H or a C1-C6 alkyl group,

said method being carried out in the presence of a compound of formula (III)



Where Y represents H; OR⁷ with R⁷ representing H, a C1-C6 alkyl group or an acyl group of formula CO-R⁴ with R⁴ meeting the preceding definition; SR⁴ or SeR⁴ with R⁴ meeting the preceding definition; or NR⁸R⁹, with R⁸ and R⁹, identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group;

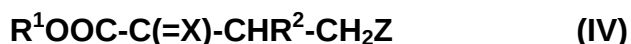
Z, identical to or different from Y, represents OR¹⁰ with R¹⁰ representing H, a C1-C6 alkyl group, or CO-R⁴ with R⁴ meeting the preceding definition; a cyclic or acyclic N(COR⁴)(COR⁴) group, with R⁴ meeting the preceding definition; or NR¹¹R¹², with R¹¹ and R¹², identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group;

Or Y and Z represent together =O;

Said method being characterized in that

The compound (II) is reacted with the compound (III) to lead to an intermediate having the structure (IV)

5



Where R^1 , R^2 , X and Z have the definition above

10 The compound (IV) thus obtained is reacted with R^4SH or a salt thereof, or R^4SeH or a salt thereof, with R^4 meeting the preceding definition, already present in the reaction medium or added during the method,

Then, upon completion of the reaction, the compound (I) or a salt thereof is isolated.

15 2. The method according to claim 1, characterized in that the reaction of the compound (IV) with R^4SH or a salt thereof, or R^4SeH or a salt thereof, with R^4 meeting the preceding definition, already present in the reaction medium or added during the method, leads to a compound having the structure (V)

20



Where R^1 , R^2 and Z have the above definition

25 A represents OH, $\text{HN-R}'$ where R' represents H, a C1-C6 alkyl group, or $\text{HN-OR}''$ where R'' represents H, a C1-C6 alkyl group, or an alkylaryl group; and B represents SR^4 or SeR^4 with R^4 meeting the preceding definition.

30 3. The method according to claim 1, characterized in that the compound (II) is reacted with hydrated or non-hydrated formaldehyde or paraformaldehyde, in a basic medium and in the presence of MeSH or a salt thereof.

35 4. The method according to claim 1, characterized in that the compound of formula (II) is reacted with a compound (III) selected from 1-[(methylsulfanyl)methyl]-piperidine, 1-[(methylsulfanyl)methyl]-pyrrolidine and 1-[(methylsulfanyl)methyl]-diethylamine.

5. The method according to claim 1, characterized in that the compound of formula (II) is reacted with a compound (III) selected from methylenedipiperidine, methylenedipyrrolidine and methylenedi(diethylamine).

5

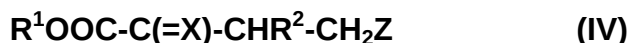
6. The method according to any one of the preceding claims, characterized in that the compound (II) is selected from the oxaloacetic acid and the pyruvic acid.

10

7. The method according to any one of the preceding claims, characterized in that, following the method, 2-oxo-4-methylthiobutanoic acid (KMB) or a salt thereof is obtained, preferably selected from the calcium, sodium, ammonium, manganese, copper, zinc and magnesium salts thereof.

15

8. A compound having the following formula (IV)



20

Where X is selected from O; N-R' Where R' represents H or a C1-C6 alkyl group; and N-OR'' where R'' represents H, a C1-C6 alkyl group, or an aryl group,

R¹ represents H or a C1-C6 alkyl group;

R² represents H, a C1-C6 alkyl group or an alkylaryl group; and

25

Z represents OR¹⁰ with R¹⁰ representing H; a C1-C6 alkyl group or CO-R⁴ with R⁴ representing H or a C1-C6 alkyl group, a cyclic or acyclic N(COR⁴)(COR⁴) group, with R⁴ representing H or a C1-C6 alkyl group; or NR¹¹R¹², with R¹¹ and R¹², identical or different, representing each or together, a C1-C6 alkyl group, or an alkylaryl group.

30

9. The compound according to claim 8, characterized in that X represents O, R² represents H and Z represents the piperidinyl group.

10. The compound having the following formula (V):

35



Where R^1 represents H or a C1-C6 alkyl group;

R^2 represents H, a C1-C6 alkyl group or an alkylaryl group;

A represents OH; HN- R' where R' represents H or a C1-C6 alkyl group;
or HN-OR'' where R'' represents H, a C1-C6 alkyl group, or an alkylaryl group;

5 B represents SR^4 or SeR^4 with R^4 representing H or a C1-C6 alkyl group;
and

Z represents OR^{10} with R^{10} representing H or a C1-C6 alkyl group or
COR⁴ with R^4 representing H or a C1-C6 alkyl group; or $NR^{11}R^{12}$, with R^{11} and
10 R^{12} , identical or different, representing each or together, a C1-C6 alkyl group, or
an alkylaryl group.

11. The compound according to claim 10, characterized in that A
represents OH, B represents SCH_3 , R^2 represents H and Z represents the
15 piperidinyl group.

12. The compound of formula (IV) according to claim 9 or compound
of formula (V) according to claim 11, where R^1 represents H.

13. A method for manufacturing D, L-methionine, D- or L-methionine,
20 D,L-2-hydroxy-4-methylthiobutanoic acid (HMTBA), D- or L-HMTBA, from 2-
oxo-4-methylthiobutanoic acid (KMB), characterized in that KMB is prepared
according to a method as defined in any one of claims 1 to 7, and then KMB is
chemically or biologically transformed into methionine or HMTBA.