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(54) Titre : PROCEDE POUR SYNTHETISER UN ACIDE GLUTAMIQUE DE L- γ -METHYLENE ET ANALOGUES
(54) Title: PROCESS FOR SYNTHESIZING L- γ -METHYLENE GLUTAMIC ACID AND ANALOGS

(57) **Abrégé/Abstract:**

A process for synthesizing substantially enantiomerically pure L-amino acids, particularly L- -methylene glutamic acid, and esters and salts thereof. The process includes the derivatization of (2S)-pyroglutamic acid, and the decyclization of the resulting derivative to form the desired end product.



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(54) Title: PROCESS FOR SYNTHESIZING L-Y-METHYLENE GLUTAMIC ACID AND ANALOGS

(57) Abstract: A process for synthesizing substantially enantiomerically pure L-amino acids, particularly L- -methylene glutamic acid, and esters and salts thereof. The process includes the derivatization of (2S)-pyroglutamic acid, and the decyclization of the resulting derivative to form the desired end product.



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PROCESS FOR SYNTHESIZING L- γ -METHYLENE
GLUTAMIC ACID AND ANALOGS

FIELD OF THE INVENTION

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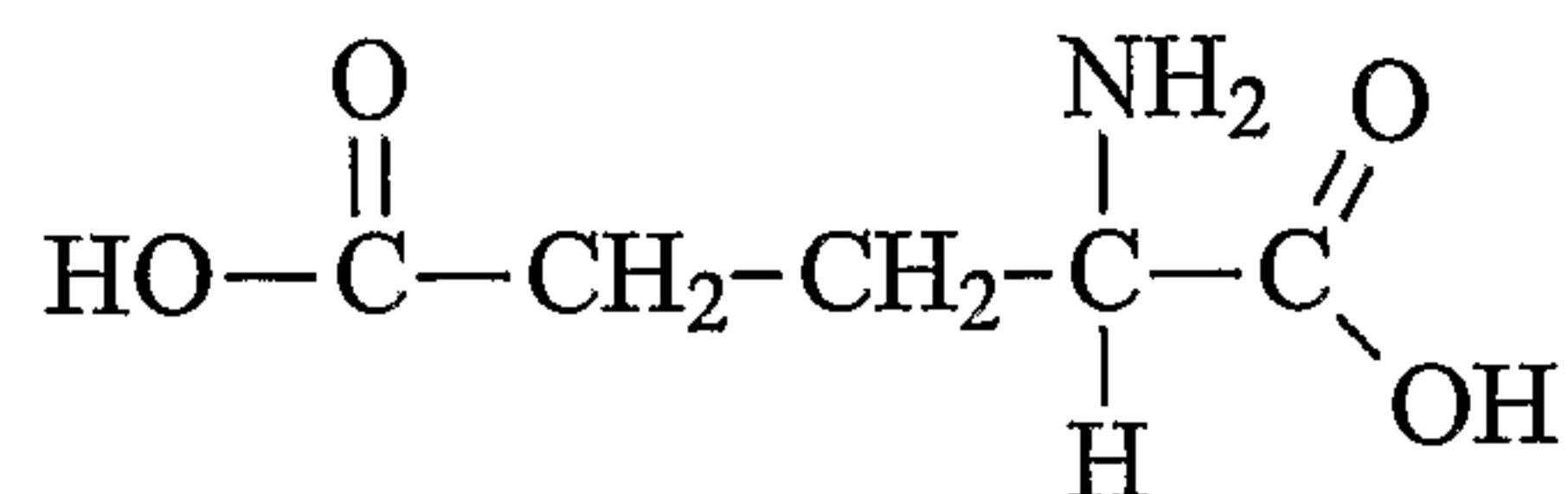
This invention relates to a process for synthesizing substantially enantiomerically pure amino acids, and will have application to a synthetic process for synthesizing the L-enantiomer of glutamic acid and substituted analogs thereof.

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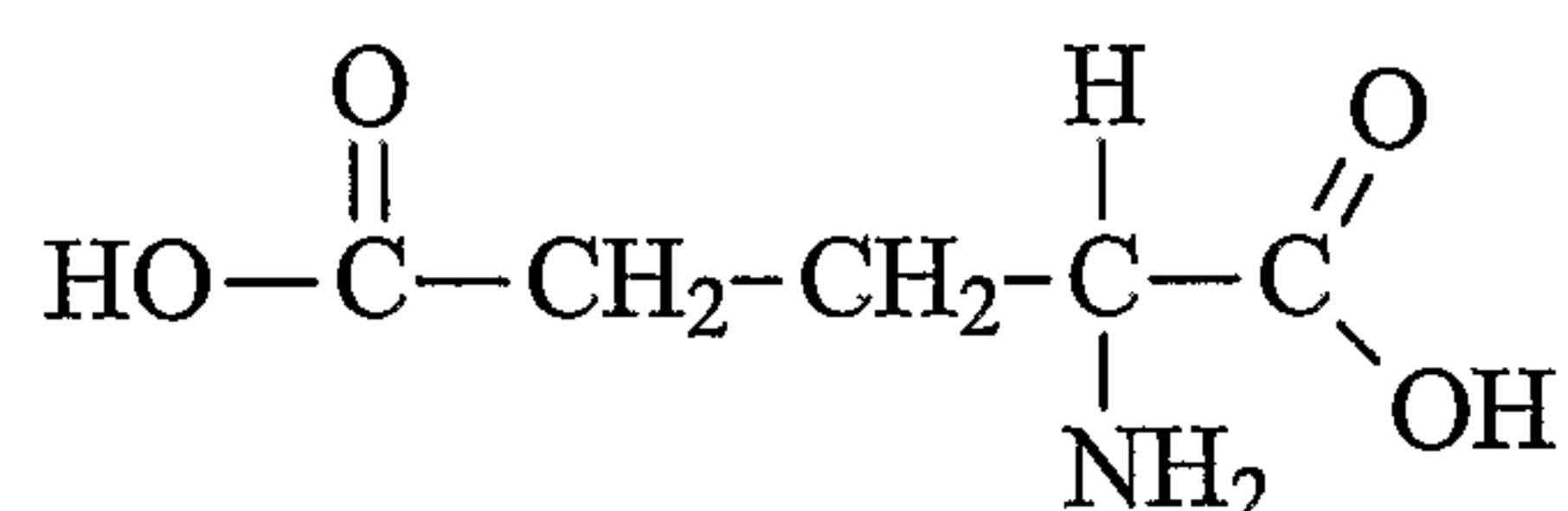
BACKGROUND OF THE INVENTION

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It is well known in biochemistry that all amino acids exist in two three dimensional forms, which are virtual mirror images of each other. The two forms are referred to in the art by the direction in which they rotate polarized light-levorotatory (L) and dextrorotatory (D), respectively left and right. Fischer representations of the structural formulas for the two enantiomers of glutamic acid are depicted below.



L-glutamic acid



D-glutamic acid

It is also well known that the L-enantiomer is the naturally occurring form of each amino acid found in nature. The L-enantiomer of each amino acid that forms a part of a drug agent is also the “active” enantiomer for medicinal purposes.

5 It is well known that antifolates which contain only the L-enantiomer of glutamic acid are much more active than those which include the D-enantiomer in a racemic mixture of the two enantiomers. The pure D-enantiomer of MDAM, as with most antifolates, is virtually inactive as a drug.

4'-methylene-4-[(2,4-diamino-3,4-dihydropteridin-6-yl)ethyl]-benzoyl-glutamic
 10 acid, also referred to as 4-deoxy-4-amino-10-deazapteroyl- -methylene glutamic acid, and as -methylene-10-deazaaminopterin (MDAM) is a well-known investigational antitumor agent. MDAM is currently undergoing human clinical trials in the United States and abroad as a treatment for cancer. MDAM and other antifolates are essentially “two-part” molecules, with a pteronic acid part and an amino acid part.

15 When L- -methylene-glutamic acid forms the amino acid part, the agent is known as L-MDAM.

-methylene-L-glutamic acid is also a well-known depolarizing agent in the rat spinal cord. This property is suggestive of potent CNS activity, which may translate into the development of agents to treat various CNS disorders.

5 -methylene-L-glutamic acid has been isolated in substantially pure form from germinated peanuts as described in US Patent 5,550,128. The free acid form of -methylene-L-glutamic acid has been synthesized starting from L-pyroglutamic acid, as shown in the Ezquerro publication, appended to the Information Disclosure Sheet that accompanies this application.

10 -methylene-L-glutamic acid, which makes up the amino acid part of L-MDAM has been previously synthesized, through a previously unpublished process from a di-protected L-pyroglutamate starting material. The pyroglutamate is available commercially, and has also been synthesized by a previously unpublished process.

SUMMARY OF THE INVENTION

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This invention involves a process for synthesizing substantially pure L-amino acids, as well as certain intermediate compounds. Primarily, the process of this invention will be used to synthesize substantially pure L-glutamic acid and intermediate compounds from which L-glutamic acid can be produced.

20

The process when employed to synthesize -methylene glutamic acid or an ester thereof includes derivatizing pyroglutamic acid or an ester thereof to add a methylene moiety; then decyclization of the pyroglutamate to form the linear glutamic acid or ester.

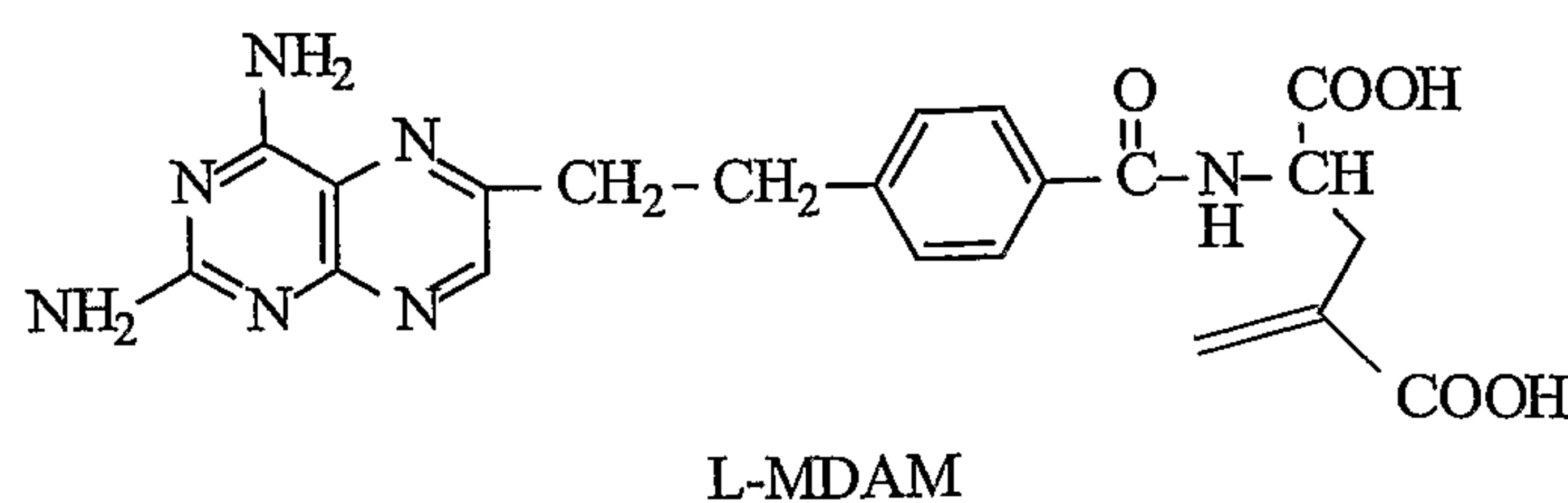
Derivatization of the pyroglutamate is accomplished by first protecting the nitrogen and carboxylic acid functional groups; then adding a γ -methylene moiety through a three-step procedure, which may be performed at or close to room temperature, and that may optionally be carried out in a single vessel. The preferred process is depicted in detail in the following Detailed Description.

It is an object of this invention to provide for a simple and improved process for synthesizing substantially pure L-glutamic acid and the like.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The preferred embodiment herein described is not intended to be exhaustive or to limit the invention to the precise reagents, reaction steps or conditions disclosed. It has been chosen and described to explain the principles of the invention, and its application and practical use to thereby enable others skilled in the art to understand its teachings.

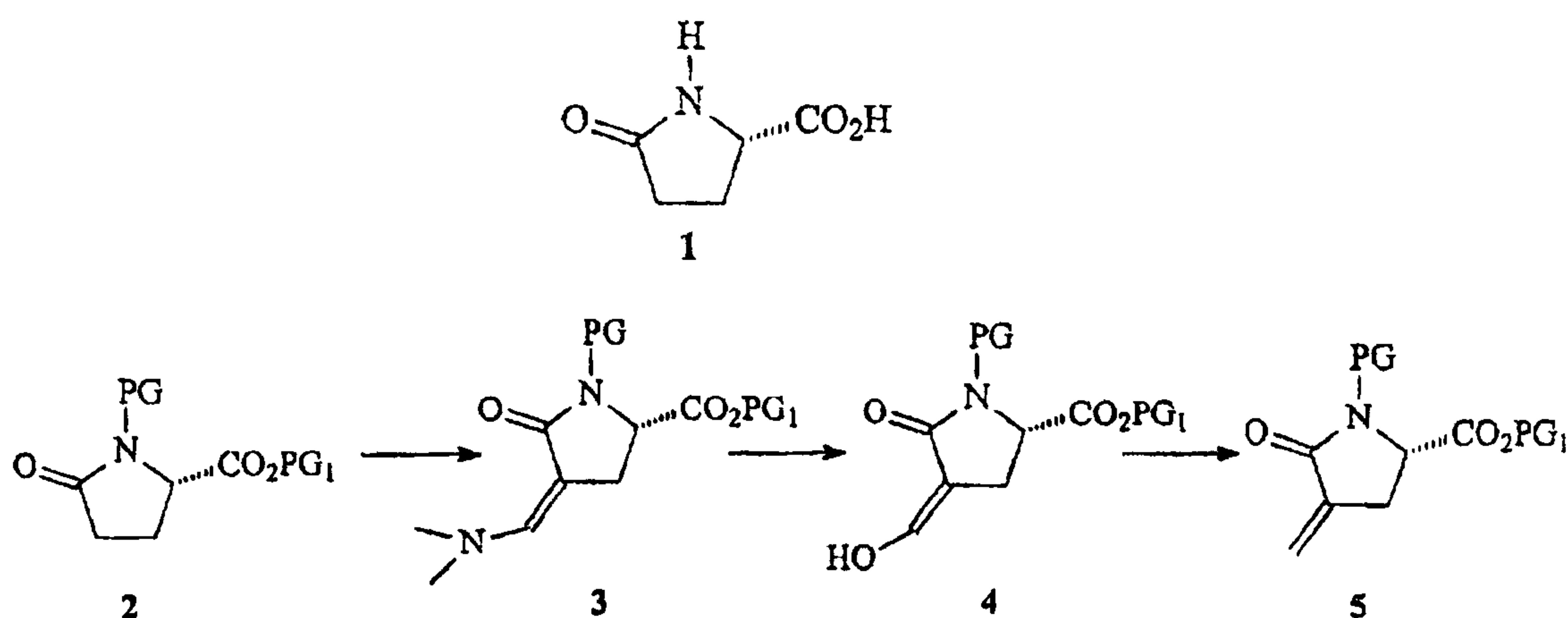
The process of this invention has as its primary objective the synthesis of substantially pure L-amino acids, most particularly the critical intermediate compound in the synthesis of the investigational anticancer drug, γ -methylene-10-deazaaminopterin (MDAM), whose structure is shown below:



As stated previously, L-MDAM is synthesized from two critical intermediates: (1) pterioic acid; and (2) -methylene-L-glutamic acid. The two intermediates are coupled via any of the known processes, with one such process being disclosed in United States Patent 4,996,207. With slight modifications in the reagents used, similar L-amino acids may be synthesized by the process of this invention.

The process for synthesizing -methylene-L-glutamic acid is depicted in Scheme 1. As shown in Scheme 1, the process includes multiple steps in converting the starting material, a pyroglutamic acid derivative or an ester thereof, to the finished product, substantially enantiomerically pure -methylene-L-glutamic acid.

SCHEME 1



Scheme 1 illustrates the process of this invention as employed to synthesize 4-methylene-L-pyroglutamic acid 5. As shown, the preferred starting reagent is L-pyroglutamic acid 1. The process includes first protecting the nitrogen and carboxylic

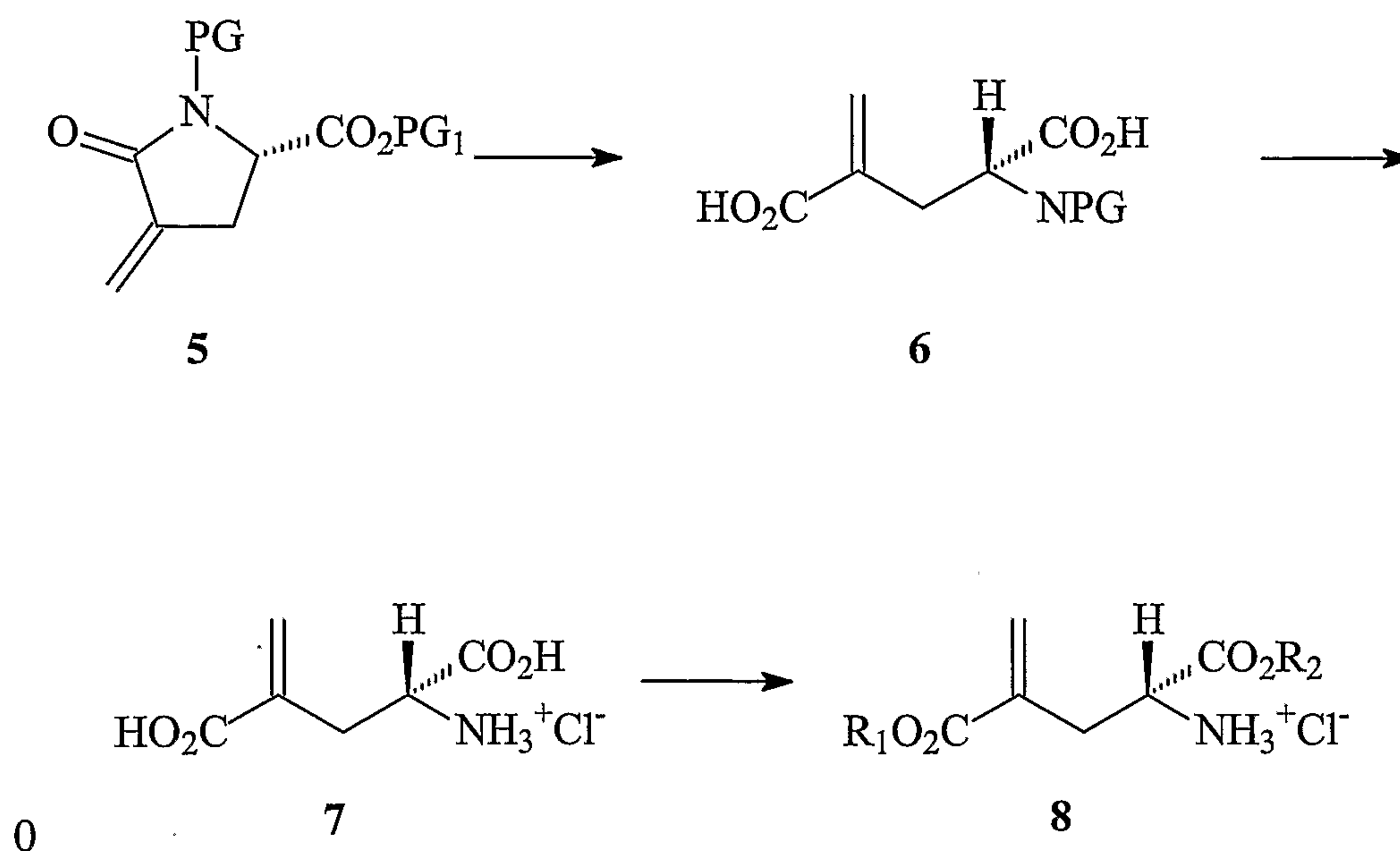
acid functional moieties to produce the diprotected pyroglutamate **2**, wherein PG and PG₁ are commonly known protecting groups for nitrogen and carboxylic acid, respectively.

Protecting groups include specific moieties for protecting, in particular, nitrogen (amino) terminal moieties and oxygen (carboxylic acid) terminal moieties. Protecting groups are well known in the art and are described in detail in *Kocienski, P., Protecting Groups*, Foundations of Organic Chemistry (Thieme, 1994); and *Greene, Wuts, Protective Groups in Organic Synthesis* (Wiley, 2d ed. 1990), and in dozens of other reference publications.

Common protecting groups for nitrogen include *t*-butoxycarbonyl (*t*-BOC or BOC), 9-fluorenyl-methoxy-carbonyl (Fmoc) and others well known in the organic synthesis arts. Common protecting groups for carboxylic acid include lower alkyls, trimethylsilyl alkyls, and others well known in the organic synthesis arts. Methods for adding protecting groups were carried out using the well-known processes in the publications cited previously.

The protected pyroglutamate **2** is then converted to a 4-enamine **3**, preferably by reacting with an amide, or most preferably a nitrogen-containing acetal. This conversion is preferably carried out at elevated temperatures, between 70 °C to 130 °C, most preferably between 105 °C and 115 °C. Enamine **3** is hydrolyzed, preferably with a strong acid, to the hydroxymethylene intermediate **4**, then reduced in a basic solution to the desired product, the diprotected 4-methylene pyroglutamate **5**. Detailed process steps and reagents, as well as preferred reaction conditions may be found in the specific examples, *infra*.

SCHEME 2



Scheme 2 illustrates the conversion of the 4-methylene pyroglutamate **5** to the preferred final product, 4-methylene-L-glutamic acid, and certain salts and esters thereof (**6-8**). A strong base is first reacted with the pyroglutamate **5**, and is then followed by acidification of the resultant mixture to effect the conversion to the N-protected linear L-glutamic acid derivative (L-Glu) **6**. Protected L-Glu **6** is deprotected by a common procedure to form L-Glu salt **7**, and the salt is then esterified to produce the final product (**8**). R_1 and R_2 are oxygen-protecting agents, preferably lower (C_1 - C_6) alkyl moieties.

The esterified L-Glu salt **8** may be used in the synthesis of L-MDAM by a coupling reaction of salt **8** to a pteronic acid derivative. The coupling reaction can be performed by any of a number of well-known procedures as outlined above.

The following specific examples illustrate the preferred process.

Example 1

Ethyl-(2S)-pyroglutamate

To a solution of L-pyroglutamic acid (5.0 g) in ethanol (150 ml) was added
5 98% sulfuric acid (0.3 g). The resulting reaction mixture was stirred at room
temperature for 48 hours. Sodium carbonate (1.5 g) was added and the stirring was
continued for an additional 1.5 hours. The suspension was filtered and the filtrate was
evaporated under reduced pressure. To the residue was added methyl *t*-butyl ether
(MTBE- 100 ml) and the resulting mixture was filtered. The filtrate was evaporated
10 under reduced pressure to yield 6.5 g of the desired product as white solid.

Example 2

Ethyl-(2S)-N-(*t*-butoxycarbonyl)pyroglutamate

15 To a solution of ethyl L-pyroglutamate (1.6 g), di(*t*-butyl)dicarbonate (2.4 g)
and triethylamine (1.6 ml) in dichloromethane (7 ml) was added dimethylaminopyridine
(DMAP- 61 mg). The resulting mixture was stirred at room temperature overnight.
The volatiles were removed under reduced pressure and the residue was taken up in
MTBE (7 ml). The solid was removed by filtration and washed with fresh MTBE.
20 The combined filtrates were washed with water (3 ml) and acidified to pH 3 with 1:9
v/v of dilute HCl. The resulting solution was washed with more water (2 x 3 ml). The
organic phase was dried over sodium sulfate and activated carbon, filtered and
evaporated under reduced pressure. The crude product was recrystallized from ether-
hexanes to yield 1.65 g of product as white needles.

25

Example 3

Ethyl-(2S)-4-(N,N-dimethylaminomethylidene)-N1-(*t*-butoxycarbonyl)pyroglutamate

A mixture of Ethyl (2S)-*N*-(*tert*-butoxycarbonyl)pyroglutamate (125 g, 486
5 mmol) and *N*, *N*-dimethyl formamide diisopropyl acetal (125 g, 713 mmol) was heated
at 105-115° C for 21 hrs. Another portion of *N*, *N*-dimethyl formamide diisopropyl
acetal (25 g, 143 mmol) was added and the solution was heated at 105-115° C for
another 4 hrs. The solution was concentrated under reduced pressure at 70° C and
dried at 70° C/ 0.3 mmHg until there is no bubbling. Crystallization from methyl *t*-
10 butyl ether (250 ml) gave the named product as a white crystal (97 g, 64%): ¹H NMR
(300Hz, CDCl₃) 7.06(t, 1H, *J* = 1.8 Hz), 4.46 (dd, 1H, *J* = 3.9, 10.5 Hz), 4.22 –
4.08 (m, 2 H), 3.32 – 3.14 (m, 1 H), 2.95 (s, 6 H), 2.89 – 2.78 (m, 1 H), 1.43 (s, 9
H), 1.21 (t, 3 H, *J* = 7.2 Hz).

15 For this example, it is recommended to use 2 equivalents of *N*, *N*-dimethyl
formamide diisopropyl acetal to each equivalent of pyroglutamate at the beginning.
After 21 hrs of heating, Another 0.2 equivalent of *N*, *N*-dimethyl formamide
diisopropyl acetal can be added if an HPLC analysis shows that the amount of starting
material is great than 1.5 %.

20

Example 4

Ethyl-(2S)-4-methylene-N1-(*t*-butoxycarbonyl)pyroglutamate

25 To a solution of ethyl (2S)-*N*-(*t*-butoxycarbonyl)-4-(dimethylaminomethylidene)

pyroglutamate (100 g, 320.1 mmol) in tetrahydrofuran (THF- 500 ml) was added 1 N

HCl (352 ml). The mixture was stirred for 2 hours at room temperature. After

separation, the organic layer was added potassium carbonate (62 g) and 37 %

formaldehyde (310 ml). The resulting solution was stirred at room temperature for 45

5 minutes, and the aqueous layer was separated. The organic layer was concentrated and

the residue was taken up in MTBE (1000 ml). The resulting mixture was washed with

water (380 ml), 20 % Na₂SO₃ (380 ml) and water (380 ml), dried (MgSO₄) and

evaporated to dryness *in vacuo* to yield the title product (71.7 g) as light yellow oil.

This crude material was used as is in the next step.

10

Example 5

4-methylene-N-(*t*-butoxycarbonyl)-L-glutamic acid

15 The crude product from Example 4 was dissolved in THF (650 ml) and to this

solution was slowly added 2M LiOH (425 ml). The resulting mixture was stirred at

room temperature for 48 hours. The aqueous layer was separated, acidified to pH 2

with 2N HCl, and extracted with ethyl acetate (2 x 500 ml). The extract was washed

with water (250 ml) and dried (MgSO₄). The dried solution was used directly in the

20 next step.

25

Example 6

4-methylene-L-glutamic acid hydrochloride

To the dried solution from Example 4 was bubbled HCl (32.5 g over a period
5 of 1 hour). The stirring was continued for an additional 1.5 hours after HCl was
stopped. The resulting white solid was filtered, washed with MTBE (100 ml) and
dried *in vacuo* to yield 31 g of substantially pure (>99%) of the title compound.

Example 7

10 Diethyl 4-methylene-L-glutamate hydrochloride

4-Methylene L-glutamic acid hydrochloride (31 g) from Example 6 was
dissolved in ethanol (310 ml). Thionyl chloride (31 ml) was added dropwise. The
resulting mixture was stirred at room temperature overnight and then refluxed for 2.5
15 hours. The reaction mixture was concentrated to dryness. The crude product was
dissolved in hot ethanol (31 ml) and to the hot solution was added anhydrous MTBE
(620 ml). The resulting mixture was kept at room temperature overnight. The crystals
of the product were filtered and dried *in vacuo* to yield 34 g of substantially pure
(>99%) title compound. The enantiomeric purity of the final product is 99.5% as
20 verified in a chiral AGP column, 4mM PBS (pH 6), isopropanol = 98.5:1.5.

What Is Claimed Is:

1. A process for synthesizing substantially enantiomerically pure 4-methylene-L-glutamic acid or an ester thereof, said process comprising the steps of:
 - a. providing a (2S)-pyroglutamic acid or an ester thereof as a starting material;
 - b. converting the starting material to a 4-enamine derivative thereof;
 - c. hydrolyzing the 4-enamine derivative to a 4-hydroxymethylidene derivative thereof;
 - d. reducing in a basic solution the 4-hydroxymethylidene derivative to a 4-methylene derivative of pyroglutamic acid or an ester thereof; and
 - e. reacting the 4-methylene pyroglutamic acid with a strong base to form linear 4-methylene glutamic acid, or esters and salts thereof.
2. The process of Claim 1 wherein step b includes reacting the starting material with an amide or an acetal.
3. The process of Claim 2 wherein step b includes reacting the starting material with an acetal at a temperature ranging from 70 C to 130 C.
4. The process of Claim 3 wherein the temperature range is from 105 C to 115 C.
5. The process of any one of Claims 1 to 4 wherein step c includes reacting the 4-enamine derivative with a strong acid.
6. The process of any one of Claims 1 to 5, wherein step d includes reacting the 4-hydroxymethylidene derivative with a carbonate salt.
7. The process of any one of claims 1 to 6, wherein the strong base is lithium hydroxide.