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(54) Title: PROCESS FOR THE PREPARATION OF ENANTIOMER-ENRICHED AMINO ACIDS

(57) Abstract: Process for the preparation of a chiral amino acid enriched in the D-enantiomer, in which a mixture of the enantiomers of the corresponding N-carbamoylamino acid is brought into contact with a D-carbamoylase with ammonia being liberated, the ammonia being removed with the aid of a bivalent metal salt of a phosphate, a monohydrogen phosphate or a dihydrogen phosphate ion. In one embodiment the enzymatic decarbamylation is carried out in the presence of a bivalent metal salt of a phosphate ion, a monohydrogen phosphate ion or a dihydrogen phosphate ion. In another embodiment the reaction mixture is brought into contact via an external loop, after separation of the solid present, with the bivalent metal salt of a phosphate ion, monohydrogen phosphate ion or dihydrogen phosphate ion. The chiral amino acid enriched in the D-enantiomer can also be obtained by enzymatically converting the corresponding hydantoin with the aid of a hydantoinase into the corresponding N-carbamoylamino acid, which is subsequently converted according to the invention into the amino acid enriched in the D-enantiomer.



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**INTERNATIONAL SEARCH REPORT**

International Application No  
PCT/NL 02/00072

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC 7 C12P13/04 C12P41/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12P C02F C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) MEDLINE, BIOSIS, EMBASE, EPO-Internal, WPI Data, PAJ		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIM G-J ET AL.: "Optimization of the enzymatic synthesis of D-p-hydroxyphenylglycine from DL-5-substituted hydantoin using D-hydantoinase and N-carbamoylase" ENZYME MICROB. TECHNOL., vol. 17, no. 1, 1995, pages 63-67, XP000951523 cited in the application abstract page 65, left-hand column, line 25-35 page 65, left-hand column, line 50-59 page 66, left-hand column, line 1 -right-hand column, line 11 --- -/--	1-10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search  6 May 2003		Date of mailing of the international search report  14. 07. 2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  van de Kamp, M

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/NL 92/00072

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>CHAO Y-P ET AL.: "Production of D-p-hydroxyphenylglycine by N-carbamoyl-D-amino acid amidohydrolase-overproducing Escherichia coli strains" BIOTECHNOLOGY PROGRESS, vol. 15, no. 4, July 1999 (1999-07), pages 603-607, XP000951585 abstract page 606, left-hand column, line 12-19 page 607, left-hand column, line 6-18 ---</p>	1-10
Y	<p>EP 0 490 396 A (NALCO CHEMIE GMBH DEUTSCHE ;VER ALUMINIUMWERKE (DE)) 17 June 1992 (1992-06-17) page 1, line 1 -page 2, line 39 ---</p>	1-10
Y	<p>WEAST R C (EDITOR): "CRC Handbook of Chemistry and Physics" 1982 , CRC PRESS INC. , BOCA RATON, FLORIDA XP002239470 page B-74 - B-78 ---</p>	1-10
A	<p>CHAO Y-P ET AL.: "One-step production of D-p-hydroxyphenylglycine by recombinant Escherichia coli strains" BIOTECHNOLOGY PROGRESS, vol. 15, no. 6, November 1999 (1999-11), pages 1039-1045, XP000952050 abstract ---</p>	4
A	<p>RUNSER S ET AL.: "D-p-hydroxyphenylglycine production from DL-5-p- hydroxyphenylhydantoin by Agrobacterium sp." APPL. MICROBIOL. BIOTECHNOL., vol. 33, no. 4, 1990, pages 382-388, XP000951524 cited in the application abstract page 385, left-hand column, line 15 -right-hand column, line 11 page 387, right-hand column, line 15-30 ---</p>	1,4
A	<p>DE 37 32 896 A (SCHULZE RETTMER RAINER ;YAWARI TOURADJ DIPL ING (DE)) 25 August 1988 (1988-08-25) column 1, line 50 -column 4, line 7 ---</p>	1
A	<p>WO 84 00885 A (ORGANON TEKNIKA CORP) 15 March 1984 (1984-03-15) cited in the application page 6, line 11 -page 8, line 10 -----</p>	1

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NL 02/00072

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10 completely

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10 (completely)

Process for the preparation of a chiral amino acid enriched in the D-enantiomer, in which a mixture of enantiomers of the corresponding N-carbamoylamino acid (e.g., produced by conversion of the corresponding hydantoin with the aid of a hydantoinase) is brought into contact with a D-carbamoylase with ammonia being liberated, characterised in that the ammonia is removed with the aid of a bivalent metal salt of a phosphate ion (phosphate salt, e.g., magnesium monohydrogen phosphate), with various additional options for process parameters.

2. Claims: 11-13 (completely)

Process for the recovery of magnesium monohydrogen phosphate wherein ammonium phosphate is contacted with a mineral acid (e.g., sulphuric acid) at a pH between 4.5 and 6.5 (e.g., between 5.5 and 6.5) and the magnesium monohydrogen phosphate is separated from the salt of ammonia and the mineral acid.

# INTERNATIONAL SEARCH REPORT

Internat	Application No
PCT/NL 02/00072	

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