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(54) Title: SIMPLE ENZYMATIC PROCESS FOR PREPARING CEFAZOLIN

(57) Abstract: A process for preparing the 7-(1-H-tetrazol-1-yl) acetamide-3-(2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl-3-
cephem-4-carboxylic acid or cefazolin, comprising catalysing the acylation of the amino β -lactam, the 7-amino-3-[5methyl-1,3,4-
thiadiazol-2-yl]thiomethyl]-3-cephem-4-carboxylic acid, of the formula with an activated acylating agent derived from acetic acid
in the presence of a biocatalyst comprising penicillin amidase, and a product of the formula I obtainable by the foregoing process
according to claims.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C12N C07D

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 465 227 B1 (RIETHORST WAANDER ET AL) 15 October 2002 (2002-10-15) the whole document ---	
A	US 5 677 141 A (ARAMORI ICHIRO ET AL) 14 October 1997 (1997-10-14) the whole document --- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FERNANDEZ-LAFUENTE R ET AL: "Chemoenzymatic One-pot Synthesis of Cefazolin from Cephalosporin C in Fully Aqueous Medium, Involving Three Consecutive Biotransformations Catalyzed by D-Aminoacid Oxidase, Glutaryl Acylase and Penicillin G Acylase" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 38, no. 26, 30 June 1997 (1997-06-30), pages 4693-4696, XP004074870 ISSN: 0040-4039 cited in the application the whole document</p> <p>---</p>	
A	<p>WON J I ET AL: "THE EFFECT OF 2-MERCAPTO-5-METHYL-1,3,4-THIADIAZOLE ON ENZYMATIC SYNTHESIS OF CEFAZOLIN" APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, CLIFTON, NJ, US, vol. 69, no. 1, January 1998 (1998-01), pages 1-9, XP001105929 ISSN: 0273-2289 cited in the application the whole document</p> <p>---</p>	
A	<p>PARK C B ET AL: "PENICILLIN ACYLASE-CATALYZED SYNTHESIS OF CEFAZOLIN IN WATER-SOLVENT MIXTURES: ENHANCEMENT EFFECT OF ETHYL ACETATE AND CARBON TETRACHLORIDE ON THE SYNTHETIC YIELD" JOURNAL OF MOLECULAR CATALYSIS. B, ENZYMATIC, ELSEVIER, AMSTERDAM,, NL, vol. 9, no. 4-6, 21 April 2000 (2000-04-21), pages 275-281, XP000989461 ISSN: 1381-1177 the whole document</p> <p>---</p>	
A	<p>JUSTIZ O H ET AL: "One-pot chemoenzymatic synthesis of 3'-functionalized cephalosporines (cefazolin) by three consecutive biotransformations in ully aqueous medium" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 62, no. 26, 1997, pages 9099-9106, XP002177920 ISSN: 0022-3263 cited in the application the whole document</p> <p>---</p> <p style="text-align: center;">-/--</p>	

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>WO 02 086143 A (BIOFERMA MURCIA S A ;SANCHEZ-FERRER ALVARO (ES); GARCIA-CARMONA FR) 31 October 2002 (2002-10-31) cited in the application the whole document -----</p>	1-39

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/12165

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6465227	B1	15-10-2002	AT 409380 B	25-07-2002
			AT 408448 B	26-11-2001
			AT 408449 B	26-11-2001
			AT 150597 A	15-12-2001
			AU 9742398 A	29-03-1999
			CN 1269830 T	11-10-2000
			WO 9913058 A2	18-03-1999
			EP 1352968 A1	15-10-2003
			EP 0991752 A2	12-04-2000
			JP 2001515715 T	25-09-2001
			US 2002192766 A1	19-12-2002
			AT 150697 A	15-04-2001
			AT 150797 A	15-04-2001
US 5677141	A	14-10-1997	AT 137803 T	15-05-1996
			CA 2032963 A1	28-06-1991
			DE 69026921 D1	13-06-1996
			DE 69026921 T2	14-11-1996
			DK 436355 T3	28-05-1996
			EP 0436355 A2	10-07-1991
			ES 2086386 T3	01-07-1996
			GR 3020071 T3	31-08-1996
			HU 58368 A2	28-02-1992
			IE 904640 A1	17-07-1991
			JP 3057759 B2	04-07-2000
			JP 4234994 A	24-08-1992
			JP 3239359 B2	17-12-2001
			JP 2000152796 A	06-06-2000
			JP 2001186894 A	10-07-2001
			KR 171897 B1	01-02-1999
			TR 27317 A	12-01-1995
WO 02086143	A	31-10-2002	WO 02085914 A2	31-10-2002
			WO 02086143 A2	31-10-2002
			EP 1379531 A2	14-01-2004
			EP 1381690 A2	21-01-2004
			US 2003104515 A1	05-06-2003
			US 2003073156 A1	17-04-2003