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(54) **An enzymatic process for preparing beta-lactams**

(57) An enzymatic process for preparing  $\beta$ -lactams, by coupling the 7-amino group of a cephalosporin nucleus with the carboxylic function of an acetic acid derivative consisting of a residue with  $\pi$ -electrons, a short spacer and a carboxylic function or derivative. The process comprises using a penicillin amidase or  $\alpha$ -amino acid esterase as free enzyme or in any suitable immobilised form; applying no or maximum 10 % organic sol-

vent, applying at least 100 mmol/l cephalosporin nucleus as long as it is soluble, otherwise 50 - 100 % of its maximum solubility is charged, applying the free organic acid in a 3 - 5 fold molar ratio or activated as ester or amide in a 1 - 3 fold molar ratio, and adjusting the pH to 5.0 - 8.0 and the temperature to 0 - 40°C.

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# EUROPEAN SEARCH REPORT

Application Number  
EP 02 07 8608

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	FERNANDEZ-LAFUENTE R ET AL.: "Dynamic reaction design of enzymic biotransformations in organic media: equilibrium - controlled synthesis of antibiotics by penicillin G acylase." BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1996 OCT) 24 ( PT 2) 139-43., vol. 24, no. 2, October 1996 (1996-10), pages 139-143, XP000989462 * the whole document *	1-8	C12P35/04 C12P35/00 C12P35/02
Y	WO 99 20786 A (DSM NV ;DOOREN THEODORUS JOHANNES GODF (NL); MOODY HAROLD MONRO (N) 29 April 1999 (1999-04-29) * the whole document * * page 10, line 17 - page 11, line 3 * * page 12, line 6-32 * * claims 1-16 *	1-8	
A	SCHROËN C G P H ET AL.: "Thermodynamically controlled synthesis of beta-lactam antibiotics. Equilibrium concentrations and side-chain properties" ENZYME AND MICROBIAL TECHNOLOGY, vol. 24, no. 8-9, June 1999 (1999-06), pages 498-506, XP00223337 * the whole document *	1,2	TECHNICAL FIELDS SEARCHED (Int.Cl.7)  C12P
A	FERNANDEZ-LAFUENTE R ET AL.: "Synthesis of antibiotics (cephaloglycin) catalyzed by penicillin G acylase: Evaluation and optimization of different synthetic approaches" ENZYME AND MICROBIAL TECHNOLOGY, vol. 19, no. 1, July 1996 (1996-07), pages 9-14, XP001098845 * the whole document *	1,2	
<p>--- -/--</p>			
<p><del>The present search report has been drawn up for all claims</del></p>			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>10 June 2003</b>	Examiner <b>van de Kamp, M</b>
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)	
D,A	EP 0 458 932 A (CONSEJO SUPERIOR INVESTIGACION) 4 December 1991 (1991-12-04) * the whole document * * example 8 *	1-3		
D,A	WO 98 04732 A (SQUIBB BRISTOL MYERS CO) 5 February 1998 (1998-02-05) * examples 1-3 * * claims 1-6 *			
A	SHAW SHYH-YU ET AL.: "Enzymatic synthesis of cephalothin by penicillin G acylase." ENZYME AND MICROBIAL TECHNOLOGY, vol. 26, no. 2-4, February 2000 (2000-02), pages 142-151, XP002233338 ISSN: 0141-0229 * the whole document *			
D,A	JUSTIZ O H ET AL.: "One-pot chemoenzymatic synthesis of 3'-functionalized cephalosporines (cefazolin) by three consecutive biotransformations in fully aqueous medium" JOURNAL OF ORGANIC CHEMISTRY, vol. 62, no. 26, 26 December 1997 (1997-12-26), pages 9099-9106, XP002233339 * the whole document *			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	KARADY S ET AL.: "The chemistry of cephamycins VI. Cleavage of the 7-amido group" TETRAHEDRON LETTERS, no. 5, 1978, pages 407-408, XP002233340 ISSN: 0040-4039 * the whole document *			
-The present search report has been drawn up for all claims				
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,A	MUNEYUKI R ET AL.: "Water-soluble esters: Useful enzyme substrates for the synthesis of beta-lactam antibiotics." CHEMISTRY AND INDUSTRY, vol. 5, 1981, pages 159-161, XP008014281 * the whole document *		
A	BRUGGINK A ET AL.: "Penicillin acylase in the industrial production of beta-lactam antibiotics" ORGANIC PROCESS RESEARCH & DEVELOPMENT, vol. 2, no. 2, March 1998 (1998-03), pages 128-133, XP002233341 * abstract *		
D,A	FERNANDEZ-LAFUENTE R ET AL.: "Equilibrium controlled synthesis of cephalothin in water-cosolvent systems by stabilized penicillin G acylase." APPL. BIOCHEM. BIOTECHNOL., vol. 27, no. 3, 1991, pages 277-290, XP008014229 * abstract *		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	NIERSTRASZ V A ET AL.: "Thermodynamically controlled synthesis of cefamandole." BIOCATALYSIS AND BIOTRANSFORMATION, vol. 17, no. 3, 1999, pages 209-223, XP008014226 * the whole document *		
-/--			
-The present search report has been drawn up for all claims			
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	<p>KASCHE V: "Mechanism and yields in enzyme-catalyzed equilibrium and kinetically controlled synthesis of beta-lactam antibiotics, peptides and other condensation products"</p> <p>ENZYME AND MICROBIAL TECHNOLOGY, vol. 8, no. 1, 1986, pages 4-16, XP008014254</p> <p>ISSN: 0141-0229</p> <p>* the whole document *</p> <p>-----</p>		
			<p>TECHNICAL FIELDS SEARCHED (Int.Cl.7)</p>
<p>-The present search report has been drawn up for all claims</p>			
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THE HAGUE		10 June 2003	van de Kamp, M
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