PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2018/055192	02.03.2018	09.03.2017

International Patent Classification (IPC) or both national classification and IPC
INV. C12N5/00

Applicant
EVONIK TECHNOCHEMIE GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Loubradou-Bourges, N Telephone No. +49 89 2399-0

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2018/055192

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 US 5 534 538 A (DRAUZ KARLHEINZ [DE] ET AL) 9 July 1996 (1996-07-09)cited in the application

D2 ANDRES SÁNCHEZ-KOPPER ET AL: "Tracking dipeptides at work-uptake and intracellular fate in CHO culture",
AMB EXPRESS,
vol. 6, no. 1, 22 December 2016 (2016-12-22), XP055365341

D3 KIM DO YUN ET AL: "Fed-batch CHO cell t-PA production and feed glutamine replacement to reduce ammonia production",
BIOTECHNOLOGY PROGRESS, WILEY-BLACKWELL PUBLISHING, INC, US,
vol. 29, no. 1, 1 January 2013 (2013-01-01), pages 165-175, XP009191145

D4 US 2003/134851 A1 (BAXTER JEFFREY H [US] ET AL) 17 July 2003 (2003-07-17)

2 Novelty and Inventive step

2.1 D1 (US 5534538), see column 2, lines 1-48 and figure 1, is considered to be the prior art closest to the subject-matter of said claims and discloses N-acyl dipeptides, in particular N-acetyl--alanyl-L-glutamine as source of glutamine in cell culture media. The dipeptide was shown to be stable under heat sterilization.

The subject-matter of claim 1 therefore differs from this known cell culture media in that either free L-glutamine or dipeptide YQ (anyAA-Q) or a combination is added and is therefore new (Article 33(2) PCT).

The application shows with experimental data (see examples) that adding free glutamine increases the relative time-related productivity and that adding acetyl AQ (AQ) leads to an improved cell-specific and time-specific productivities as well as relative titers.

The problem to be solved by the present invention may be regarded as the provision of improved cell culture media.

The solution to this problem proposed in claim1 is considered as involving an inventive step (Article 33(3) PCT) since the skilled person would not have envisaged without hindsight that the addition of either free L-glutamine or a further dipeptide providing the free L-Glutamine (exemplified by AQ) would have led to an improvement in the cell culture.

Claims 2-11 are dependent on claim 1 and as such also meet the requirements of the PCT with respect to novelty and inventive step.

Claims 12 (use of the cell culture media) and claims 13-14 (process) are use and method directly related to the cell culture medium and thus also meet the requirements of the PCT with respect to novelty and inventive step.

2.2 The skilled person is aware that free L-glutamine is unstable and also that alternative sources sources of glutamine are available.

D2-D4 relate to such alternative sources:

further dipeptides comprising Q as GQ tested in D2 (Sanchez-Kopper), see abstract,
commercially available glutamine-containing peptide (GlutaMAX), pyruvate, glutamate, wheat gluten hydrolysate tested in D3 (Kim), see abstract and Table 2,
and also N-acetyl-L-glutamine available in D4 (US 2003/134851) which is shown to be stable, see figures 1-3.

None of these compounds is however comprised in the scope of claim 1.

2.3 The description is however broader than the scope of the claims.