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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

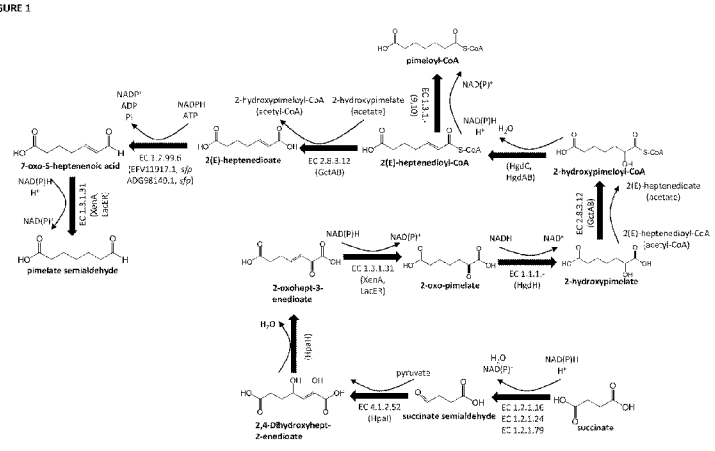
— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

[Continued on next page]

(54) Title: METHODS OF PRODUCING 7-CARBON CHEMICALS VIA PYRUVATE AND SUCCINATE SEMIALDEHYDE ALDOL CONDENSATION

FIGURE 1



(57) Abstract: This document describes biochemical pathways for producing one or more of pimelic acid, 7-aminoheptanoic acid, 7-hydroxyheptanoic acid, heptamethylenediamine and 1,7-heptanediol by forming one or two terminal functional groups, comprised of carboxyl, amine or hydroxyl groups, in a C7 aliphatic backbone substrate produced from succinate semialdehyde or pyruvate. These pathways, metabolic engineering and cultivation strategies described herein rely on the aldol condensation of succinate semialdehyde and pyruvate.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/077430

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 fees, this Authority did not invite payment of additional fees.

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.:

1-33(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/077430

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/18 C12P13/001 C12P13/002 C12P7/42 C12P7/44
ADD.

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)
C12P

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 practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/068944 A2 (CELEXION LLC [US]; BAYNES BRIAN M [US]; GEREMIA JOHN MICHAEL [US]) 17 June 2010 (2010-06-17)	1
Y	page 1, paragraph [0001] page 9, lines 1-3, paragraph [0031] page 10, lines 11-22, paragraph [0032] page 24, paragraph [0057] - page 28, paragraph [0066] page 44, paragraph [0086] - page 48, paragraph [0090]; claims 30,31	1-33

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family 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 application or patent 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	Date of mailing of the international search report
14 August 2014	10/11/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mateo Rosell, A

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Week 201304 Thomson Scientific, London, GB; AN 2012-R78158 XP002728487, & WO 2012/177721 A1 (GENOMATICA INC) 27 December 2012 (2012-12-27) WO2012177721 contains 667 pages; see page 30, line 10 - page 31, line 24; fig 12, 20,21; claims	1-33
Y	IZUMI ET AL: "Structure and Mechanism of HpcG, a Hydratase in the Homoprotocatechuate Degradation Pathway of Escherichia coli", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 370, no. 5, 14 June 2007 (2007-06-14), pages 899-911, XP022117287, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2007.05.006 cited in the application page 899, left-hand column, paragraph 1 - page 900, left-hand column, paragraph 1; figures 1,3	1
Y	REA ET AL: "Structure and Mechanism of HpcH: A Metal Ion Dependent Class II Aldolase from the Homoprotocatechuate Degradation Pathway of Escherichia coli", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 373, no. 4, 3 October 2007 (2007-10-03), pages 866-876, XP022285563, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2007.06.048 cited in the application abstract page 866, left-hand column, paragraph 1 - page 867, left-hand column, paragraph 2; figures 1-2,7	1
Y,P	WO 2013/096898 A2 (INVISTA NORTH AMERICA SARL [US] INVISTA TECH SARL [LU]) 27 June 2013 (2013-06-27) abstract; figures 1, 10	1-33
Y,P	WO 2013/003744 A2 (INVISTA TECHONOLOGIES S A R L [CH]; PEARLMAN PAUL S [US]; CHEN CHANGLI) 3 January 2013 (2013-01-03) abstract; figures 1,10	1-33
2 E	WO 2014/105788 A2 (INVISTA NORTH AMERICA SARL [US]) 3 July 2014 (2014-07-03) claims 37,39,40,42,43	1-33
1		

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/077430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010068944 A2	17-06-2010	AU 2009324422 A1	07-07-2011
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		EP 2726627 A2	07-05-2014
		US 2014242655 A1	28-08-2014
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		WO 2014105788 A2	03-07-2014

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-33(partially)

A method for enzymatic biosynthesis of pimelic acid from succinate semialdehyde and pyruvate via aldol condensation and enzymatically forming two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in said backbone, thereby forming the product.

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2. claims: 1-33(partially)

A method for enzymatic biosynthesis of 7-aminoheptanoate from succinate semialdehyde and pyruvate via aldol condensation and enzymatically forming two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in said backbone, thereby forming the product.

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3. claims: 1-33(partially)

A method for enzymatic biosynthesis of 7-hydroxyheptanoate from succinate semialdehyde and pyruvate via aldol condensation and enzymatically forming two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in said backbone, thereby forming the product.

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4. claims: 1-33(partially)

A method for enzymatic biosynthesis of heptamethylenediamine from succinate semialdehyde and pyruvate via aldol condensation and enzymatically forming two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in said backbone, thereby forming the product.

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5. claims: 1-33(partially)

A method for enzymatic biosynthesis of 1,7-heptanediol from succinate semialdehyde and pyruvate via aldol condensation and enzymatically forming two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in said backbone, thereby forming the product.

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6. claims: 34-40(partially)

A recombinant host comprising at least one exogenous nucleic acid encoding an 4-hydroxy-2-oxopimelate aldolase, a 2-hydroxyhepta-2,4-dienedioate hydratase, an enoate reductase, a 2-hydroxyglutarate dehydrogenase, a glutaconate CoA-transferase, a 2-hydroxyglutaryl-CoA dehydratase, an enoyl-CoA reductase or an enoyl-[acp] reductase, said host producing pimeloyl-CoA.

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7. claims: 34-40(partially)

A recombinant host comprising at least one exogenous nucleic acid encoding an 4-hydroxy-2-oxopimelate aldolase, a 2-hydroxyhepta-2,4-dienedioate hydratase, an enoate reductase, a 2-hydroxyglutarate dehydrogenase, a glutaconate CoA-transferase, a 2-hydroxyglutaryl-CoA dehydratase, and a carboxylate reductase, said host producing pimelate semialdehyde.