# PATENT COOPERATION TREATY

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INTERNATIONAL SEARCHING AUTHORITY

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# 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/EP2018/072191 | 16.08.2018 | 17.08.2017 |

International Patent Classification (IPC) or both national classification and IPC
INV. C07K14/62 A61K38/28

Applicant
NOVO NORDISK A/S

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<br>P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040<br>Fax: +31 70 340 - 3016 | see form PCT/ISA/210 | Gurdjian, Didier<br>Telephone No. +31 70 340-0 |

**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 | 2-10 |
| | No: | Claims | 1, 11-15 |
| Inventive step (IS) | Yes: | Claims | 4, 7, 9 |
| | No: | Claims | 1-3, 5, 6, 8, 10-15 |
| Industrial applicability (IA) | Yes: | Claims | 1-15 |
| | No: | Claims | |

2. Citations and explanations

**see separate sheet**

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

Reference is made to the following documents:

D1 WO 00/69901 A2 (XENCOR INC [US]) 23 November 2000 (2000-11-23)

D2 TANG L ET AL: "Structural consequences of the B5 histidine-tyrosine mutation in human insulin characterized by X-ray crystallography and conformational analysis",
BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY, US,
vol. 38, no. 37, 21 August 1999 (1999-08-21), pages 12041-12051,
XP002777856,
ISSN: 0006-2960

D3 WO 2005/054291 A1 (NOVO NORDISK AS [DK]; KJELDSEN THOMAS BOERGLUM [DK]; ANDERSEN ASSER SL) 16 June 2005 (2005-06-16)cited in the application

D4 WO 96/15804 A1 (LILLY CO ELI [US]) 30 May 1996 (1996-05-30)cited in the application

D5 WO 2007/096431 A1 (NOVO NORDISK AS [DK]; FYNBO CHARLOTTE HARKJAER [DK]; JONASSEN IB [DK];) 30 August 2007 (2007-08-30)

D6 WO 2006/008238 A1 (NOVO NORDISK AS [DK]; JESSEN CLAUS ULRICH [DK]; HANSEN LOUIS BRAMMER [) 26 January 2006 (2006-01-26)

D7 WO 2012/015692 A2 (SMARTCELLS INC [US]; LANCASTER THOMAS M [US]; MURIKIPUDI SYLAJA [US];) 2 February 2012 (2012-02-02)

D8 WO 2009/112583 A2 (NOVO NORDISK AS [DK]; NIELSEN PETER KRESTEN [DK]; HUBALEK FRANTISEK [D) 17 September 2009 (2009-09-17)

D9 QING-XIN HUA ET AL: "A Conserved Histidine in Insulin Is Required for the Foldability of Human Proinsulin : STRUCTURE AND FUNCTION OF AN ALA B5 ANALOG",
JOURNAL OF BIOLOGICAL CHEMISTRY,
vol. 281, no. 34, 25 August 2006 (2006-08-25), pages 24889-24899,
XP055468704,
US
ISSN: 0021-9258, DOI: 10.1074/jbc.M602617200

D10 B. TER BRAAK ET AL: "Classifying the adverse mitogenic mode of action of insulin analogues using a novel mechanism-based genetically engineered human breast cancer cell panel", ARCHIVES OF TOXICOLOGY, vol. 88, no. 4, 1 April 2014 (2014-04-01), pages 953-966, XP055521551, DE ISSN: 0340-5761, DOI: 10.1007/s00204-014-1201-2

D11 BAS TER BRAAK ET AL: "Mammary gland tumor promotion by chronic administration of IGF1 and the insulin analogue AspB10 in the p53R270H/+WAPCre mouse model", BREAST CANCER RESEARCH, CURRENT MEDICINE GROUP LTD, GB, vol. 17, no. 1, 18 February 2015 (2015-02-18), page 14, XP021214496, ISSN: 1465-5411, DOI: 10.1186/S13058-015-0518-Y

The first invention of the present application relates to the provision of improved acylated insulin derivatives e comprises B5Y or B5F, further comprises B26G or B26A, wherein said substituent comprising an acyl group is attached to B28K, B26K or B29K, where the acyl is selected from the group consisting of: lithocholic acid, 1,16-hexadecanedioic acid, 1,18-octadecanedioic acid, 1,20-eicosanedioic acid, tetrazole-C16, tetrazole-C17, tetrazole C18 and tetradecanoic acid.

The present application substantiates that the B5Tyr and B5Phe mutations have highly stabilizing effect on the insulin zinc hexamer.

It substantiates the insulin derivatives of the present inventions, having a specific acylation as defined in claim 4-11 are capable of reducing blood glucose levels, but have improved effects on processes related to lipid metabolism, such as less weight gain, less increase in body fat mass and increased lowering of liver TG, as well as improved endothelial function relative to a matched comparator.

The second invention of the present application relates to a method for determining selectivity of an insulin compound comprising the following steps:

• measuring the maximal AKT phosphorylation induced by said insulin compound relative to human insulin

• measuring the maximal ERK activation induced by said insulin compound relative to human insulin,

wherein the ERK/AKT ratio is less than 1.

The present application substantiates that the insulin derivatives of the present invention have a lower submaximal effect on ERK activation (phosphorylation) than on AKT activation (phosphorylation), when compared to the effect of human insulin.

## Re Item V

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

### 1. Novelty

D1 discloses the provision the non-naturally occurring protein with insulin activity useful for treating type 1 and type 2 diabetes, comprising amino acid substitutions as compared to native human insulin and having enhanced stability. It comprises a substitution at A3, A5-A7, A11, A15, A16, A19, A20, B2, B7, B15, B19, B22. It comprises an amino acid positions B5 and B14 (preferably **B5-F, B5-W,** B14-F, B14-W, B14-Y or B14-I and forms a hexamer in the absence of a phenolic preservative. It discloses the specific insulins : GIVEQCCTSI CSLYQLENYC NFVNQ**F**LCGS HLVEALYLVC GERGFFYTPK , GIVEQCCTSI CSLYQLENYC NFVNQ**F**LCGS HLVEWLYLVC GERGFFYTPK or GIVEQCCTSI CSLYQLENYC NFVNQ**F**LCGS HLVEFLYLVC GERGFFYTPK, comprsing the B5 Phe mutations. It discloses the provision of acetylated variants with specific modification of tyrosyl residues, such as for introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. N acetylimidizol and tetranitromethane may be used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. The human insulin analog act as antidiabetics. (see abstract; page 26, line 24 - line 28; claims 1-21; figures 3,4)

D10 (XP055521551) discloses the the adverse mitogenic mode of action of insulin analogues. Insulin analogues are widely used in clinical practice. Modifications on the insulin molecular structure can affect the affinity and activation towards two closely related receptor tyrosine kinases: the insulin receptor (INSR) and the insulin-like growth factor 1 receptor (IGF1R). While most insulin analogues primarily activated the

INSR, the mitogenic activation pattern of glargine was highly similar to IGF1 and insulin AspB10, known to bind IGF1R and induce carcinogenesis. D10 discloses. D10 discloses that insulin derivatives have a lower submaximal effect on ERK activation (phosphorylation) than on AKT activation (phosphorylation), when compared to the effect of human insulin. D10 discloses a method for determining selectivity of an insulin compound comprising the following steps:

• measuring the maximal AKT phosphorylation induced by said insulin compound relative to human insulin

• measuring the maximal ERK activation induced by said insulin compound relative to human insulin,

wherein the ERK phosphorylation is lower that the AKT phosphorylation, implying that the ratio is less than 1.( see the abstract, and fig.3)

D11 ( XP021214496) discloses the effects of Mammary gland tumor promotion by chronic administration of IGF1 and the insulin analogue AspB10. It studied the role of insulin analogues , with four different insulin (-like) molecules: normal insulin, insulin glargine, insulin X10 (AspB10) or insulin-like growth factor 1 (IGF1). D11 discloses that insulin derivatives have a lower submaximal effect on ERK activation (phosphorylation) than on AKT activation (phosphorylation), when compared to the effect of human insulin.D11 discloses a method for determining selectivity of an insulin compound comprising the following steps: • measuring the maximal AKT phosphorylation induced by said insulin compound relative to human insulin • measuring the maximal ERK activation induced by said insulin compound relative to human insulin, wherein the ERK phosphorylation is lower that the AKT phosphorylation, implying that the ratio is less than 1.( see the abstract, and fig.3)

In view of D1, the subject-matter of claims 1,11-14 is not new . In view of D10,D11, the subject-matter of claim 15 is not new

## 2. Inventive step

D2 discloses the structural consequences of the B5 histidine --> tyrosine mutation in human insulin characterized by X-ray crystallography and conformational analysis. . The B5 His --> Tyr mutant human insulin was constructed to see if the tyrosine side chain would mimic the effect of phenol binding in the hexamer and induce the R-state.

As expected, in the presence of phenol or resorcinol, the B5 Tyr hexamers adopt the fully helical (R6) conformation. This suggests that packing constraints around residue B5 Tyr result in the observed structural rearrangements. Thus, rather than promoting the R-state in a manner analogous to phenol, the mutation appears to destabilize the T-state. D2 highlight the role of B5 His in determining hexamer conformation and in mediating crystal packing interactions, properties that are likely be important in vivo. It discloses that this mutation promotes crystallized hexamer packing o in the insulin storage vesicles. It discloses that he mutants can be applied as slow acting insulins. (see abstract; page 12051, left-hand column, paragraph 1 - paragraph 2)

D3 discloses the provision of mono-acylated human insulin analogue - useful in treatment of diabetes mellitus, has extended duration of action. The single-chain insulin may be acylated with an acyl group which may be a linear or branched carboxylic acid . Examples of fatty acids are capric acid, lauric acid, tetradecanoic acid (myristic acid), pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, dodecanoic acid, tride- canoic acid, and tetradecanoic acid. The acyl group may also be a lipophilic substituent selected from the group comprising CH3 (CH2) nCO-, wherein n is 4 to 24, such as CH3 (CH2) 6CO-, CH3 (CH2) 8CO-, CH3 (CH2) 10CO-, CH3 (CH2) 12CO-, CH3 (CH2) 14CO-, CH3 (CH2) 16CO-, CH3 (CH2) 18CO-, CH3 (CH2) 2oC0- and CH3 (CH2) 22CO-. In one embodiment of the invention the acyl group is a straight-chain or branched alkane a, c3-dicarboxylic acid. In another embodiment of the invention the acyl group has the formula HOOC (CH2) tCO- wherein t is an integer of from 2 to 24. The acyl group may by a lithocholic acid as lithocholoyl or choloyl. The ingle-chain insulin is acylated in at least one ly sine group in the single-chain insulin molecule, e.g. being acylated in B29Lys. (see the abstract, page 11, line 6 - page 12, line 7; claims 1-34)

D5 discloses an insulin derivative comprising a parent insulin and a substituent, wherein the substituent is attached either to an [epsilon]-amino group of a Lys residue present in the A-chain of the parent insulin at position A8, A9, A10, A12, A14, A15, A17, A18, A21 , A22, A23 or A24 or to an [epsilon] -amino group of a Lys residue in the B-chain of the parent insulin at position B1 , B2, B3, B4, B20, B21 or B22 provided that when B3 is Lys, then B29 is not Glu, wherein the acyl group is 5-[alpha] lithocholic acid or 5-[beta] lithocholic acid., or wherein the acyl group is 5-[alpha] or 5-[beta] isomers of cholic acid, hyocholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid or cholanic acid, or wherein the acyl group is a 5-[alpha] or 5-[beta] isomer of dehydrolithocholic acid. D5 discloses that the acyl

group is connected to a lysine residue using an amino acid linker. According to this aspect the acyl group is advantageously connected to a lysine residue via a [gamma]- or an [alpha]-glutamyl linker, or via a [beta]- or an [alpha]-aspartyl linker, or via an [alpha]-amido-[gamma]- glutamyl linker, or via an [alpha]-amido-[beta]-aspartyl linker. (see abstract; page 9 line 7- page 13 line 35, claims 1-11)

D7 discloses recombinantly expressed insulin polypeptide wherein the polypeptide comprises an amino acid sequence of SEQ ID NO:26 (A-peptide) and an amino acid sequence of SEQ ID NO:27 (B-peptide), wherein Xaa at position B5 is His , wherein Xaa at position B26 is Tyr or Ala , wherein Xaa at position B28 is Pro, Ala, Lys, Leu, Val, or Asp , wherein Xaa at position B29 is Lys, Pro or Glu , wherein Xaa at position, and/or B(26-30) is missing. (see abstract; claims 1-32)

D8 discloses an insulin analog where the A-chain of the insulin analog comprises at least one mutation relative to the parent insulin, where one mutation is in position A14 which is substituted to an amino acid selected from Lys, Glu, Arg, Asp, Pro, Gln and His; and the B-chain of the insulin analog comprises at least two mutations relative to the parent insulin. The B-chain of the insulin analogue comprises at least two mutations relative to the parent insulin, wherein two or more mutations are in the form of deletions of the amino acids in positions B27, B28, B29 and B30, or a combination of a deletion of the amino acid in position B30 and a substitution of an amino acid selected from the amino acid substitutions in position: B24 to Gly or His, B25 to His, B26 to Gly, Glu or Lys, B27 to Gly, Glu or Lys and B28 to Asp, His, Gly, Lys or Glu; which is selected from the group consisting of: desB27, desB28, desB29, desB30 human insulin desB27, desB30 human insulin desB28, desB29, desB30 human insulin. (see the abstract; claims 1-8)

D9 discloses that a conserved histidine in insulin is required for the foldability of human proinsulin: structure and function of an ALAB5 analog. It discloses that nascent long-range interactions by His(B5) facilitate alignment of Cys(A7) and Cys(B7) in protein-folding intermediates; its conservation thus reflects mechanisms of oxidative folding rather than structure-function relationships in the native state. ( see the abstract)

D2 is considered to be the closest prior at for the subject-matter of claim 11.It differs by the lack of further mutations outside B5.

The problem is the further provision of insulin B5Tyr intermediates and derivatives. In view of D7 , the skilled person would have obviously provided further B5 insulin intermediates comprising , at position B26 Tyr or Ala, at position B28 Lys , at position B29 Lys.In view of D8 and D7 he would have provided the desB27, desB28, desB29, desB30 deletion. The subject-matter of claim 11 is not inventive.

D1 is considered to be the closest art for the subject-matter of claims 2,3 , 5-6, 8,10 It differs by the by the lack of mutations in B26-29, and A14. The problem is the provision of further acylated insulin variants. In view of D7 he would have provided at position B26 Tyr or Ala, at position B28 Lys , at position B29 Lys. In view of D8 he would have provided mutant A14E, and desB27, desB28, desB29, desB30 deletions. In view of D5 he would have provided at the Lys B28, or B29K an acyl group is 5-[alpha] lithocholic acid or 5-[beta] lithocholic acid., or wherein the acyl group is 5-[alpha] or 5-[beta] isomers of cholic acid, hyocholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid or cholanic acid, or wherein the acyl group is a 5-[alpha] or 5-[beta] isomer of dehydrolithocholic acid. In view od D5 he would have provided an acyl group is connected to a lysine residue using an amino acid linker. According to this aspect the acyl group is advantageously connected to a lysine residue via a [gamma]- or an [alpha]-glutamyl linker, or via a [beta]- or an [alpha]-aspartyl linker, or via an [alpha]-amido-[gamma]- glutamyl linker, or via an [alpha]-amido-[beta]-aspartyl linker. The subject-matter of claims 2,3 , 5-6, 8 ,10is not inventive in the sense of art. 56 EPC.

However, the skilled person would have no reasonable expectation if success to foresee that the specific acylation of claims 4,7, and 9 would in synergy with the substitution B5Tyr or B5 Phe , provide a highly stabilizing effect on the insulin zinc hexamer, and be surprisingly capable of reducing blood glucose levels, have improved effects on processes related to lipid metabolism, such as less weight gain, less increase in body fat mass and increased lowering of liver TG, as well as improved endothelial function relative to a matched comparator. The subject-matter of claims 4,7, and 9 appears to be inventive .

## Re Item VIII

**Certain observations on the international application**

For the assessment of the present claims 13-15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The present search was directed towards all claims. However, when entering in the regional phase, an objection related to non-unity could be raised , by separating the subject-matter of claims 1-14 as the first invention, and the subject-matter of claim 15 as the second invention.

Moreover, related to the subject-matter of claim 15, the specific conditions on how to measure the selectivity of insulin compounds compared to human insulin is lacking. Its subject-matter lacks an essential technical element and is hence unsupported.