

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MYLAN PHARMACEUTICALS INC.,

Petitioner,

v.

NOVO NORDISK A/S,

Patent Owner.

Case No. IPR2023-00722

Patent No. 8,536,122

**DECLARATION OF DR. CHRISTOPHER J. SOARES, PH.D.,
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 8,536,122**

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TABLE OF ABBREVIATIONS

Full Name of Cited Reference	Abbreviation
U.S. Patent No. 8,536,122	'122 patent
Accardo, <i>Physicochemical Properties of Mixed Micellar Aggregates Containing CCK Peptides and Gd Complexes Designed as Tumor Specific Contrast Agents in MRI</i> , 126 J. AM. CHEM. SOC. 3097 (2004)	Accardo
Adelhorst, <i>Structure-Activity Studies of Glucagon-like Peptide-1*</i> , 269(9) J. BIO. CHEM. 6275 (1994)	Adelhorst
Albericio, <i>Orthogonal Protecting Groups for N(alpha)-amino and C-terminal carboxyl functions in Solid-Phase Peptide Synthesis</i> , 55(2) BIOPOLYMERS 123 (2000)	Albericio
Amblard, <i>Fundamentals of Modern Peptide Synthesis</i> , in PEPTIDE SYNTHESIS AND APPLICATIONS 3 (John Howl ed., 2005)	Amblard
Autio, <i>Mini-PEG Spacering of VAP-I-Targeting ⁶⁸Ga-DOTAVAP-PI Peptide Improves PET Imaging of Inflammation</i> , 1 EJNMMI RSCH. 1 (2011)	Autio
Baggio, <i>A Recombinant Human Glucagon-Like Peptide (GLP)-1–Albumin Protein (Albugon) Mimics Peptidergic Activation of GLP-1 Receptor–Dependent Pathways Coupled with Satiety, Gastrointestinal Motility, and Glucose Homeostasis</i> , 53(9) DIABETES 2492 (2004)	Baggio
Bhattacharya, <i>Crystallographic Analysis Reveals Common Modes of Binding of Medium and Long-Chain Fatty Acids to Human Serum Albumin</i> , 303 J. MOL. BIOL. 721 (2000)	Bhattacharya
U.S. Patent No. 6,514,500	Bridon
Cistola, <i>Carbon 13 NMR Studies of Saturated Fatty Acids Bound to Bovine Serum Albumin. I. The filling of Individual Fatty Acid Binding Sites</i> , 262(23) J BIOL CHEM. 10971 (1987)	Cistola I
Cistola, <i>Carbon 13 NMR Studies of Saturated Fatty Acids Bound to Bovine Serum Albumin. II. Electrostatic Interactions in Individual Fatty Acid Binding Sites</i> , 262(23) J BIOL CHEM. 10980 (1987)	Cistola II

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Clodfelter, <i>Effects of Non-Covalent Self-Association on the Subcutaneous Absorption of a Therapeutic Peptide</i> , 15(2) PHARM. RSCH. 254 (1998)	Clodfelter
Nulf, <i>DNA Assembly using Bis-Peptide Nucleic Acids (bisPNAs)</i> , 30(13) NUCLEIC ACIDS RES.2782 (2002)	Corey 2002
Dong, <i>Glucagon-Like Peptide-1 Analogs with Significantly Improved in vivo Activity</i> , in PEPTIDES: THE WAVE OF THE FUTURE (Michal Lebl et al. eds., 2001)	Dong
Drucker, <i>Development of Glucagon-Like Peptide-1-Based Pharmaceuticals as Therapeutic Agents for the Treatment of Diabetes</i> , 7 CURRENT PHARM. DESIGN 1399 (2001)	Drucker 2001
Drucker, <i>Discovery, Characterization, and Clinical Development of the Glucagon-Like Peptides</i> , 127(12) J. CLIN. INVEST. 4217 (2017)	Drucker 2017
U.S. Patent No. 5,359,030	Ekwuribe
U.S. Patent No. 7,576,050	Greig
Veronese, <i>Introduction and Overview of Peptide and Protein Pegylation</i> , 54 ADVANCED DRUG DELIVERY REVIEWS 453 (2002)	Veronese
Holst, <i>The Incretin Approach for Diabetes Treatment Modulation of Islet Hormone Release by GLP-1 Agonism</i> , 53 (suppl. 3) DIABETES S197 (2004)	Holst 2004
Holz, <i>Glucagon-Like Peptide-1 Synthetic Analogs: New Therapeutic Agents for Use in Treatment of Diabetes Mellitus</i> , 10 CURRENT MED. CHEM. 2471 (2003)	Holz
Kenyon, <i>¹³C NMR Studies of the Binding of Medium-Chain Fatty Acids to Human Serum Albumin</i> , 35(3) J LIPID RES. 458 (1994)	Kenyon
Kim, <i>Development and Characterization of a Glucagon-Like Peptide 1-Albumin Conjugate: The Ability to Activate the Glucagon-Like Peptide 1 Receptor In Vivo</i> , 52(3) DIABETES 751 (2003)	Kim

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(continued)

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Kiso, <i>Amide Formation, Deprotection, and Disulfide Formation in Peptide Synthesis</i> , in PEPTIDES: SYNTHESIS, STRUCTURES, AND APPLICATIONS 39 (Bernd Gutte ed., 1995)	Kiso
U.S. Patent No. 6,268,343	Knudsen Patent
Knudsen, <i>Potent Derivatives of Glucagon-Like Peptide-1 with Pharmacokinetic Properties Suitable for Once Daily Administration</i> , 43(9) J MED CHEM. 1664 (2000)	Knudsen 2000
Knudsen, <i>GLP-1 Derivatives as Novel Compounds for the Treatment of Type 2 Diabetes: Selection of NN2211 for Clinical Development</i> , 26(7) DRUGS OF THE FUTURE (2001)	Knudsen 2001
Knudsen, <i>Glucagon-Like Peptide-1: The Basis of a New Class of Treatment for Type 2 Diabetes</i> , 47(17) J MED CHEM. 4128 (2004)	Knudsen 2004
Kurtzhals, <i>Albumin Binding of Insulins Acylated with Fatty Acids: Characterization of the Ligand-Protein Interaction and Correlation between Binding Affinity and Timing of the Insulin Effect in vivo</i> , 312 BIOCHEM J. 725 (1995)	Kurtzhals
LEHNINGER PRINCIPLES OF BIOCHEMISTRY (David L. Nelson et al. eds., 4th ed. 2005)	Lehninger
Luu, <i>Automated Multiple Peptide Synthesis: Improvements in Obtaining Quality Peptides</i> , 47 INT'L J. PEPTIDE PROTEIN RSCH 91 (1996)	Luu 1996
Markussen, <i>Soluble, Fatty Acid Acylated Insulins Bind to Albumin and Show Protracted Action in Pigs</i> , 39(3) DIABETOLOGIA 281 (1996)	Markussen
Meinenhofer, <i>Peptide Synthesis: A Review of the Solid-Phase Method</i> , in HORMONAL PROTEINS AND PEPTIDES (Choh Hao Li ed., 1973)	Meinenhofer
Ozempic prescribing information (Oct. 2022)	Ozempic Label

TABLE OF ABBREVIATIONS
(continued)

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Pasut, <i>Protein, Peptide and Non-Peptide Drug PEGylation for Therapeutic Application</i> , 14 EXPERT OPINION ON THERAPEUTIC PATENTS 859 (2004)	Pasut
Peters, ALL ABOUT ALBUMIN BIOCHEMISTRY, GENETICS, AND MEDICAL APPLICATIONS (1996)	Peters
Rando, <i>Functional Incorporation of Synthetic Glycolipids into Cells</i> , 77(5) PROC. NATL. ACAD. SCI. USA 2510 (1980)	Rando 1980
Roberts, <i>Chemistry for Peptide and Protein PEGylation</i> , 54(4) ADV DRUG DELIV. REV. 459 (2002)	Roberts
WO 03/74005	Sato
Schmidt, <i>Fatty Acylation of Proteins</i> , 988 BIOCHIMICA ET BIOPHYSICA ACTA 411 (1989)	Schmidt 1989
Sewald, PEPTIDES: CHEMISTRY AND BIOLOGY (2002)	Sewald
Tonsgard, <i>Binding of Straight-Chain Saturated Dicarboxylic Acids to Albumin</i> , 82 J. CLIN. INVEST. 1567 (1988)	Tonsgard 1988

I. INTRODUCTION

1. My name is Christopher J. Soares, Ph.D. I have been retained by Mylan Pharmaceuticals Inc. (“Mylan”). I understand that Mylan intends to petition for *inter partes* review (“IPR”) of U.S. Patent No. 8,536,122 (“the ’122 patent”), Ex. 1001, which is assigned to Novo Nordisk A/S (“Patent Owner”). As part of the petition, I understand Mylan will request that the United States Patent and Trademark Office (“USPTO”) cancel claims 1-2, 4-11, 13, and 15 of the ’122 patent (“the challenged claims”) as unpatentable. I submit this expert declaration to address and support Mylan’s IPR petition for the challenged claims of the ’122 patent.

II. QUALIFICATIONS AND BACKGROUND

A. Education and Experience; Prior Testimony

2. I am currently the Founder/CEO of AcheRx, a peptide therapeutics company investigating diseases linked to the perception and transmission of neuropathic pain.

3. I received a Bachelor of Science in Chemistry from the University of Bombay, India in 1980, a Master of Science in Chemistry from Hampton University in 1984, and a Ph.D. from the University of California, Davis in 1989, where my research focused on the total synthesis of natural products using a novel chemical rearrangement.

4. Since receiving my Ph.D., I have gained extensive experience as a peptide medicinal chemist, with additional expertise in drug discovery and development related to peptide therapeutics. In 1989, I started my career in peptide and protein design as a postdoctoral fellow at The Scripps Research Institute, where I remained until 1992.

5. From 1992 to 1998, I worked at Amylin Pharmaceuticals, Inc., a biotechnology company focused on discovering new medicines for metabolic disorders including diabetes and obesity, first as a Senior Research Associate, Process Chemistry, then as a Staff Scientist, Research Chemistry, and finally as a Senior Staff Scientist, Research Chemistry.

6. After working as a Research Scientist in medicinal chemistry at CombiChem Inc. (later DuPont Pharmaceuticals Inc.) from 1998 to 2002, I returned to Amylin, where I worked until 2010, beginning as a Scientific Investigator, Research Chemistry, then as a Senior Scientific Investigator, Research Chemistry, and finally as an Associate Director, Research. While at Amylin, I incorporated my knowledge of peptide structure and protein design into drug discovery and development. As a co-program leader for various peptide-based drug discovery efforts I successfully nominated three candidates for clinical development. Of these candidates two were in the calcitonin family of hormones that include amylin and calcitonin gene-related peptide ("CGRP") and the third

was a glucagon-like peptide 1 (“GLP-1”) agonist developed as a chemically superior analogue of the approved drug Byetta. One other drug molecule I designed was a dual amylin and GLP-1 agonist that resulted in the initiation of a program to develop hybrid peptide hormones. I also lead a discovery program to identify oral and long-acting analogues and formulations of GLP-1 agonists towards the end of my association with Amylin Pharmaceuticals Inc. I successfully coordinated the projects, collaborated with various research and development groups (e.g., *in vitro* and *in vivo* assay development, bioanalytical assay development, regulatory strategy, budgets, staffing), and produced publications and patents from each project.

7. From 2010-2013 I served as a consulting R&D director at Avryll Therapeutics Inc., developing liposome formulations of a therapeutic for cartilage repair. In 2013, I founded AcheRx, LLC, a company focusing on the design and development of peptide therapeutics for neuropathic pain and migraine. While at AcheRx, I have been involved with the discovery of a novel series of peptide CGRP antagonists, for which I have received multiple compound and method-of-use patents. I have also designed a novel dual CGRP-amylin peptide agonist as a potential new weight-loss therapeutic for which I have an issued patent. Additionally, I have been awarded a Small Business Innovative Research (SBIR) grant from the NIH/NINDS for developing a slow-release formulation of a CGRP

antagonist. The lead peptide has been chosen by the NIH Preclinical Screening Platform for Pain (PSPP) program as a potential compound to develop as a non-addictive pain therapeutic.

8. Throughout my career, I have produced numerous peer-reviewed publications related to peptide design and therapeutics. I have also been named as the sole or co-inventor on several issued patents related to my work.

9. I am a member of the American Association of Pharmaceutical Sciences (AAPS), the American Peptide Society (APS) and the European Peptide Society (EPS). At the national meeting of the AAPS in 2007, I gave a presentation on the discovery and development of a novel peptide therapeutic for metabolic diseases at the 'Peptide Leads to the Drugs of Tomorrow' section. The lead peptide in this program was licensed to a large pharmaceutical company after Phase II clinical studies.

10. In the previous four years, I have not provided testimony in any proceedings.

11. My qualifications are further described on my curriculum vitae, at Exhibit 1023.

B. Legal Standards and Materials Reviewed

12. In preparing and forming my opinions set forth in this declaration, I have been informed of the relevant legal principles. I have applied my

understanding of those principles in forming my opinions. My understanding of those principles is summarized below.¹

13. I have been informed that Mylan bears the burden of proving unpatentability by a preponderance of the evidence. Counsel has informed me that this “preponderance of the evidence” standard means Mylan must show that unpatentability is more probable than not. I have taken these principles into account when forming my opinions.

14. I understand that my opinions regarding unpatentability are from the viewpoint of a person having ordinary skill (“POSA”) in the field of technology of the patent as of the priority date of the ’122 patent. I have also been informed that claims should be given their plain and ordinary meaning in light of the intrinsic evidence (i.e., the specification and the prosecution history) from the perspective of a POSA.

¹ In performing my analysis and reaching my opinions and conclusions, I have been informed of and been advised to apply various legal principles relating to unpatentability, which I set forth herein. In setting forth these legal standards, it is not my intention to testify about the law. I only provide my understanding of the law, as explained to me by counsel, as a context for the opinions and conclusions I am providing in this declaration.

15. I also understand that the concept of patent obviousness involves four factual inquiries: (1) the scope and content of the prior art; (2) the differences between the claimed invention and the prior art; (3) the level of ordinary skill in the art; and (4) secondary considerations of non-obviousness.

16. I was informed that claims of a patent may be found obvious if, in view of the prior art, a POSA would have been motivated to combine the teachings of the prior art to arrive at the claimed subject matter with a reasonable expectation of success in doing so. I further understand that absolute predictability of success is not required for patent claims to be found obvious.

17. I understand that when there is some recognized reason to solve a problem, and there are a finite number of identified, predictable and known solutions, a POSA has good reason to pursue the known options within their technical grasp. If such an approach leads to the expected success, it is likely not the product of innovation but of ordinary skill and common sense. I understand that any need or problem known in the field of endeavor at the time of invention or addressed by the patent can provide a reason for combining prior art elements to arrive at the claimed subject matter.

18. I was also informed that the claims of a patent may be found obvious if, in view of the prior art, the claimed subject matter involves combinations of known elements that would have been obvious to try.

19. I also understand from counsel that claims of a patent directed to chemical compounds may, in some instances, require what's referred to as a lead compound obviousness analysis. I was informed that this lead compound analysis involves a two-part obviousness test. First, Mylan must show that a POSA would have selected a prior art compound, or compounds, as a "lead compound" (i.e., a compound that would be a natural choice for further development efforts). I understand a POSA could identify multiple compounds as "lead compounds" so long as a POSA would have had a reason to select each proposed lead compound.

20. Second, Mylan must show that a POSA would have been motivated to modify the lead compound(s) to arrive at the claimed compound. I understand the motivation to modify a lead compound can come from any number of sources and does not need to be explicitly disclosed in the prior art.

21. A list of the materials I considered, in addition to my experience, education, and training, in providing the opinions contained herein is attached as Appendix A.

C. Scope of Work

22. I have been retained by Mylan as a technical expert to provide various opinions regarding the '122 patent. I am being compensated for my work in this case at a rate of \$900 per hour plus expenses. My compensation is in no way tied to the outcome of this case or to the content of this declaration. I do not have any

current or past affiliation with Patent Owner, any affiliates of Patent Owner presently known to me, or the named inventors of the '122 patent.

III. PERSON OF ORDINARY SKILL IN THE ART

23. I understand my analysis is to be conducted from the perspective of a POSA as of the priority date of the '122 patent. I have also been informed by counsel that in defining a POSA, the following factors may be considered: (1) the educational level of the inventors; (2) the type of problems encountered in the art; (3) prior art solutions to those problems; (4) rapidity with which innovations are made; and (5) sophistication of the technology and educational level of active workers in the field. Further, I understand a POSA is generally skilled in the relevant art (i.e., the subject matter claimed and described in the patent).

24. A POSA would have understood the prior art references cited herein and would have the capability to draw inferences from them. It is understood that, to the extent necessary, a POSA may collaborate with one or more other POSAs and/or others with relevant knowledge for one or more aspects with which the others may have expertise, experience, and/or knowledge. Additionally, a POSA could have had a lower level of formal education than described in the following definitions if the person has a higher degree of experience.

25. I understand that Dr. Flatt has provided an opinion, with which I agree, that the following definition of a POSA should apply with respect to the challenged claims of the '122 patent.

26. The subject matter of the claims of the '122 patent falls within the medicinal chemical and pharmacological arts and encompasses the skills, education, and expertise of a team of individuals working together to develop and formulate GLP-1 analogues, as well as to use the GLP-1 analogues to treat patients having type-2 diabetes or related conditions. Such a team would have included individuals with an M.D., Pharm.D., or doctoral degree(s) in chemistry, biochemistry, pharmaceutics, pharmaceutical sciences, chemical engineering, biochemical engineering, or related fields, with at least two years of experience in developing therapeutic peptides or proteins, and experience with the development, design, manufacture, formulation, or administration of therapeutic peptides or proteins, and the literature concerning protein or peptide formulation and design or diabetes treatments.

27. Alternatively, the POSA (1) would be a highly skilled scientist lacking an M.D., Pharm.D., or doctoral degree, but (2) (a) would have more than five years of experience in the area of developing therapeutic proteins or peptides and/or (b) would have experience with the development, design, manufacture, formulation, or administration of therapeutic agents for diabetes, and the literature

concerning protein or peptide formulation and design or diabetes treatments. In either case, a higher educational level could substitute for some amount of the requisite experience.

28. Such a team also would have included persons with an appropriate level of skill in medicinal synthetic chemistry, including the synthesis and chemical modification of peptides or proteins.

29. With respect to claim 13 of the '122 patent, the team would have included an individual with a Ph.D. in chemistry, biochemistry, pharmaceuticals, pharmaceutical sciences, chemical engineering, biochemical engineering or related fields, with at least two years of experience in the formulation of therapeutic peptides or proteins.

30. With respect to the subject matter of claim 15 of the '122 patent, the team would have included an individual with an M.D. and experience treating patients having type-2 diabetes or related conditions.

31. As explained above, and as is evident from my CV, I met the qualifications of a POSA as of the priority date of the '122 patent.

IV. THE '122 PATENT

32. I have read the '122 patent, including its claims, which is titled "Acylated GLP-1 Compounds." I understand that the '122 patent has 16 issued claims, of which only claim 1 is an independent claim. The '122 patent lists Jesper

Lau, Florencio Zaragoza Doerwald, Paw Bloch, and Thomas Kruse Hansen as inventors.

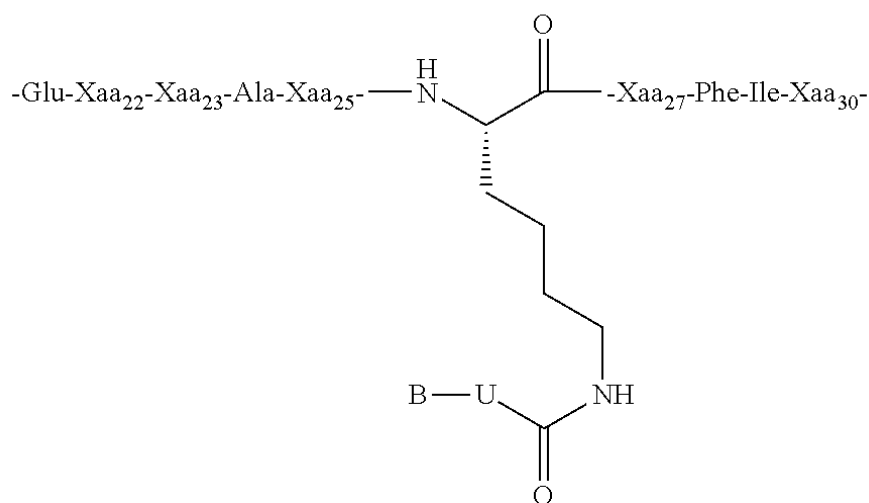
33. The '122 patent was filed on March 5, 2012, as U.S. Patent Application No. 13/412,283, a continuation of application No. 11/908,834, filed as application No. PCT/EP2006/060855 on March 20, 2006, and now U.S. Patent No. 8,129,343. The '122 patent also claims priority to U.S. Provisional Application No. 60/664,497, filed on March 23, 2005, as well as European application No. 05102171, filed March 18, 2005.

34. I understand the earliest priority date to which the challenged claims of the '122 patent are entitled is March 18, 2005, the filing date of European application No. 05102171. Therefore, I understand that references that predate March 18, 2005, are prior art to the '122 patent. To the extent Patent Owner later asserts and/or proves that the challenged claims are entitled to an earlier priority or invention date, I reserve the right to supplement this declaration.

35. The '122 patent has one independent claim, which recites:

1. A compound of formula II (SEQ ID No. 3):

Xaa₇-Xaa₈-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Xaa₁₆-Ser-Xaa₁₈-Xaa₁₉Xaa₂₀-



-Trp-Leu-Xaa₃₃-Xaa₃₄-Xaa₃₅-Xaa₃₆-Xaa₃₇-Xaa₃₈,

wherein

Xaa₇ is L-histidine, D-histidine, desamino-histidine, 2-amino-histidine, β -hydroxy-histidine, homohistidine, N ^{α} -acetyl-histidine, α -fluoromethyl-histidine, α -methyl-histidine, 3-pyridylalanine, 2-pyridylalanine, or 4-pyridylalanine;

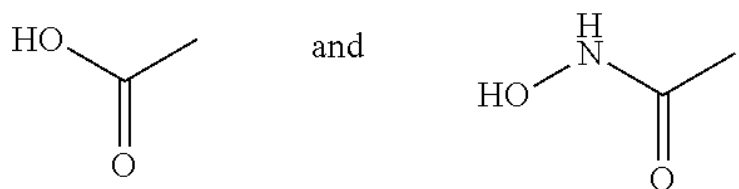
Xaa₈ is Gly, Val, Leu, Ile, Lys, Aib, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

Xaa₁₆ is Val or Leu; Xaa₁₈ is Ser, Lys, or Arg; Xaa₁₉ is Tyr or Gln; Xaa₂₀ is Leu or Met; Xaa₂₂ is Gly, Glu, or Aib; Xaa₂₃ is Gln, Glu, Lys, or Arg; Xaa₂₅ is Ala or Val; Xaa₂₇ is Glu or Leu;

Xaa₃₀ is Ala, Glu, or Arg²; Xaa₃₃ is Val or Lys; Xaa₃₄ is Lys, Glu, Asn, or Arg; Xaa₃₅ is Gly or Aib; Xaa₃₆ is Arg, Gly, Lys, or is absent; Xaa₃₇ is Gly, Ala, Glu, Pro, Lys, or is absent; Xaa₃₈ is Lys, Ser, amide, or is absent;

and where U is a spacer selected from [various spacers]

where B is an acidic group selected from



36. Claim 2 depends from independent claim 1 and recites a narrower set of possible amino acids than is recited in claim 1 (designated by “Xaa”).

37. Claim 4 depends from independent claim 1 and recites that the “GLP-1 analog comprises Aib⁸ or Gly⁸ in position 8 of the GLP-1(7-37) sequence.” Claim 5 depends from claim 4 and specifies that the amino acid at position 8 is Aib (i.e., aminoisobutyric acid).

38. Claims 6 and 7 depend from independent claim 1 and specify that the GLP-1 analogue comprises no more than six (claim 6) or three (claim 7) “amino

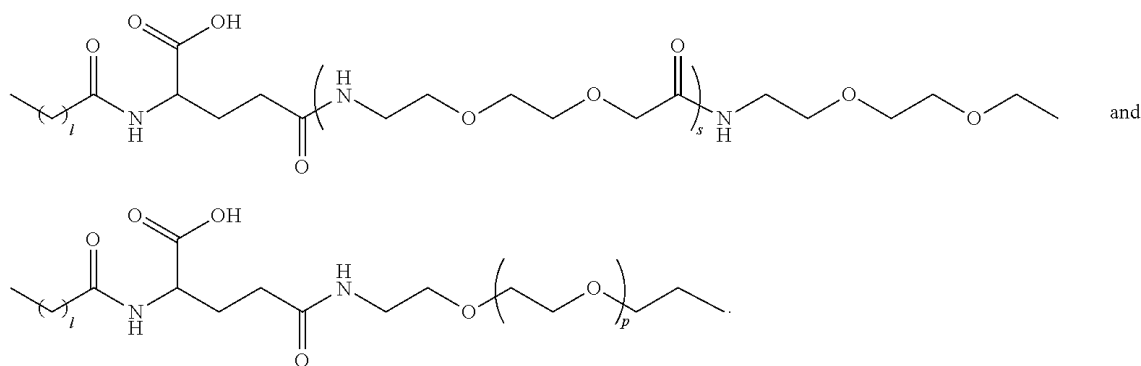
² I understand that while claim 1 originally recited “Xaa₃₃ is Ala, Glu, or Arg,” a certificate of correction was issued by the USPTO on April 4, 2015, to revise this claim term to read “Xaa₃₀ is Ala, Glu, or Arg.”

acid residues which have been exchanged, added or deleted as compared to GLP-1(7-37).”

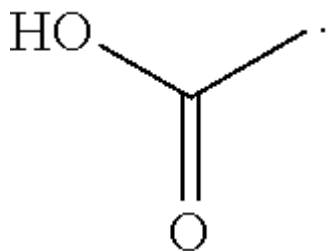
39. Claim 8 depends from independent claim 1 and recites that the “GLP-1 analog comprises only one lysine residue.”

40. Claim 9 depends from independent claim 1 and recites that the GLP-1 analogue “according to claim 1 ... is Aib⁸, Arg³⁴-GLP-1(7-37) or Aib^{8,22}, Arg³⁴-GLP-1(7-37).”

41. Claim 10 depends from independent claim 1 and further specifies that “U is a spacer selected from” the following:



42. Claim 11 depends from claim 10 and requires “B,” as recited in independent claim 1, to be:



43. Claim 13 depends from independent claim 1 and recites “[a] pharmaceutical composition comprising a compound according to claim 1, and a pharmaceutically acceptable excipient.”

44. Claim 15 depends from independent claim 1 and recites “[a] method for treating hyperglycemia and/or type 2 diabetes in a subject, said method comprising administering to a subject in need of such treatment an effective amount of a GLP-1 analog according to claim 1.”

V. CLAIM CONSTRUCTION

45. I have been informed that claim terms should be given their plain and ordinary meaning in light of the intrinsic evidence (i.e., the specification and the prosecution history) from the perspective of a POSA. I also understand that while extrinsic evidence (e.g., scientific publications) may be considered when interpreting the meaning of claim terms in some circumstances, it is generally given less weight than intrinsic evidence.

46. There are no terms relevant to my opinions that require construction beyond the plain and ordinary meaning.

VI. SUMMARY OF OPINIONS

47. I understand that additional experts are offering opinions on behalf of Mylan regarding obviousness of the challenged claims of the ’122 patent. In particular, I understand that Dr. Peter Flatt, Ph.D., and Dr. John Bantle, M.D., have

explained that as of the priority date, the POSA would have been motivated to pursue an improved GLP-1 analogue that required less frequent administration, which would have been preferred by patients and likely improved compliance with treatment regimens.³ To this end, I understand that Dr. Flatt has opined that the POSA would have been motivated to select liraglutide as a lead compound for further development, and that, given the goal of less frequent administration, a POSA would have found it obvious to modify liraglutide to make semaglutide (a GLP-1 analogue encompassed by all challenged claims of the '122 patent). Specifically, I understand that Dr. Flatt opines that the POSA would have been motivated to modify liraglutide, with a reasonable expectation of successfully improving its half-life while retaining at least the same antidiabetic properties, by (1) substituting aminoisobutyric acid ("Aib") for alanine ("Ala") at position 8 of the peptide chain; (2) adding a spacer with two [2-(2-amino)ethoxy]ethoxy acetic acid ("AEEA") units between the existing glutamyl spacer-fatty acid substituent and the lysine ("Lys") at position 26 ("Lys²⁶") of the peptide chain; and (3) acylating the peptide with a C₁₈ fatty diacid rather than the C₁₆ monocarboxylic acid used in liraglutide.

³ I understand that Dr. Paul Dalby, Ph.D., is also offering expert opinions on behalf of Mylan, particularly as to formulation issues pertinent to the challenged claims.

48. For purposes of this declaration, I assume the POSA's motivation to select liraglutide as a lead compound. I further explain from the perspective of a medicinal chemist why it is also my opinion that a POSA would have been motivated to add a di-AEEA spacer between Lys²⁶ and the glutamyl-fatty acid substituent of liraglutide to further improve the compound's half-life and hydrophilicity/solubility with a reasonable expectation of success. It is also my opinion that the POSA would have been motivated to modify liraglutide by using a C₁₈ fatty diacid in place of the C₁₆ fatty monoacid used in liraglutide with a reasonable expectation of success.

49. First, as to the addition of a di-AEEA spacer, as I explain in Section IX.A, this would have been an obvious application of routine principles known for decades in the prior art. Indeed, the art taught the successful use of polyethylene glycol ("PEG")-based molecules as spacer groups between fatty acids and peptides to increase the peptide's half-life by improving albumin binding. And studies from as early as 1980 reported the benefits of using the PEG-based AEEA moiety specifically. AEEA spacers were also expressly used in GLP-1 analogues to increase peptide half-life with successful results, including the use of two AEEA molecules together (i.e., a "di-AEEA" spacer), as used later in semaglutide, in prior-art embodiments. The POSA would have thus had ample motivation to use one or more PEG-based molecules, and specifically the AEEA moiety, as a spacer

to increase the length of the linkage between Lys²⁶ and the existing glutamic acid linker used in liraglutide. A POSA would have understood that the increased length imparted by a di-AEEA spacer would enhance albumin binding by virtue of the space it creates between the peptide and albumin, ensuring the two would not interfere with one another. And the POSA would have had a reasonable expectation that it could implement such a spacer (indeed, the chemistry itself was routine) to improve the half-life or hydrophilicity/solubility of the resulting peptide.

50. Second, the POSA would have also been motivated to acylate the peptide with a C₁₈ fatty diacid rather than the C₁₆ monocarboxylic acid as used in liraglutide. Such a modification would have been considered conservative (i.e., with limited effect on liraglutide's therapeutic performance), but presented a recognized improvement in half-life. Indeed, GLP-1 analogues acylated with a C₁₈ fatty diacid at the same lysine residue as liraglutide were also expressly disclosed in the prior art, including in embodiments that demonstrated an improved half-life over liraglutide. The prior art also taught that the binding pocket for fatty acids with respect to albumin is about 18 carbons long, and thus the POSA would have been further motivated to use a C₁₈ fatty diacid over less ideal, shorter or longer potential options. For these reasons and those discussed further in Section IX.B below, the POSA would have had ample motivation to modify liraglutide to use a

C₁₈ fatty diacid in this manner, with a reasonable expectation that the resulting peptide could be easily synthesized, and would have a suitably long half-life and desirable antidiabetic properties.

51. Finally, in my opinion, the challenged claims of the '122 patent would have also been an obvious result of routine experimentation, arrived at through testing known options for known variables or by optimization of known result-effective variables. With the motivation to develop a GLP-1 analogue with an improved half-life, the POSA's experimentation would have been to test options that were small, incremental modifications that would not adversely deviate from—and in fact were intended to improve upon—the known chemical and biological properties of the parent drug liraglutide. With these motivations in mind, the POSA would be faced with only a narrow set of practical options for each of the discrete modifications made, which could be quickly and easily tested through routine screening processes, leading to semaglutide through no more than trivial trial and error experimentation.

VII. BACKGROUND ON DRUG SYNTHESIS

A. Drug-Structure Optimization

52. Broadly speaking, drug-compound development can be grouped into two distinct categories: (1) drug discovery and (2) drug-structure optimization.

53. Drug discovery, as I understand it, involves the exploration and discovery of brand-new compounds not already known to exist, and the discovery process may involve many unpredictable developments, or require the application of new techniques, as it progresses. For instance, the discovery of GLP-1 in the 1970s and 80s through (at the time) novel recombinant DNA approaches is one example of drug discovery. *See, e.g.,* Ex. 1018 (Drucker 2017).

54. Drug-structure optimization, on the other hand, involves taking a known compound and making modifications to its structure in routine and predictable ways. When a compound's structure-activity relationship ("SAR")—the relationship between certain aspects of a compound's structure and its impact on the pharmaceutical properties such as efficacy, toxicity, hydrophilicity, lipophilicity, duration of action, etc.—is relatively unknown, experiments may be conducted to alter various aspects of the compound's structure to better characterize the SAR.

55. But once a compound's SAR is well understood, the drug-compound optimization process can be easier. For instance, if one portion of a compound is known to degrade due to its hydrophilicity, drug developers would recognize the need to modify the compound to incorporate a hydrophobic element. Such optimizations may still require investigation and testing by the drug developer because it may be unknown which modification within the group (e.g., known

hydrophobic elements) will ultimately serve its intended purpose best. However, drug developers would turn to options or strategies known in the art to resolve those issues with an expectation of relatively predictable results. Indeed, drug developers may expect many of the known options within the group would work to some degree, and it is simply finding which particular modification serves the drug developer's purposes best.

56. Accordingly, once a compound's SAR is well understood, drug-structure optimization involves applying known options or techniques, which can be a predictable and routine path in many cases. In the case of peptides specifically, the drug development and optimization process has benefited from technological advances.

57. Indeed, peptides have been particularly fortunate in the compound-synthesis space, as numerous machines and techniques have been developed since the 1960s to effectively automate the peptide-synthesis processes. *See, e.g.*, Ex. 1037 (Luu 1996) at 1-7 ("A large number of peptides can be produced by using ABIMED's automated multiple peptide synthesizer"); Ex. 1028 (Greig) at 11:43-13:46 (describing GLP-1 analogues that "can be prepared using any number of chemical polypeptide synthesis techniques well known to those of ordinary skill in the art including solution methods and solid phase methods," and summarizing known methods).

B. Peptide Synthesis

58. Peptides are made of amino acid units called “residues” bound sequentially together in a polymeric chain. Ex. 1036 (Lehninger) at 26. This chain is formed by amide linkages, or “peptide bonds,” which are formed by simple chemical reactions. *Id.* See generally Ex. 1059 (Kiso) (reviewing basics of chemical reactions involved).

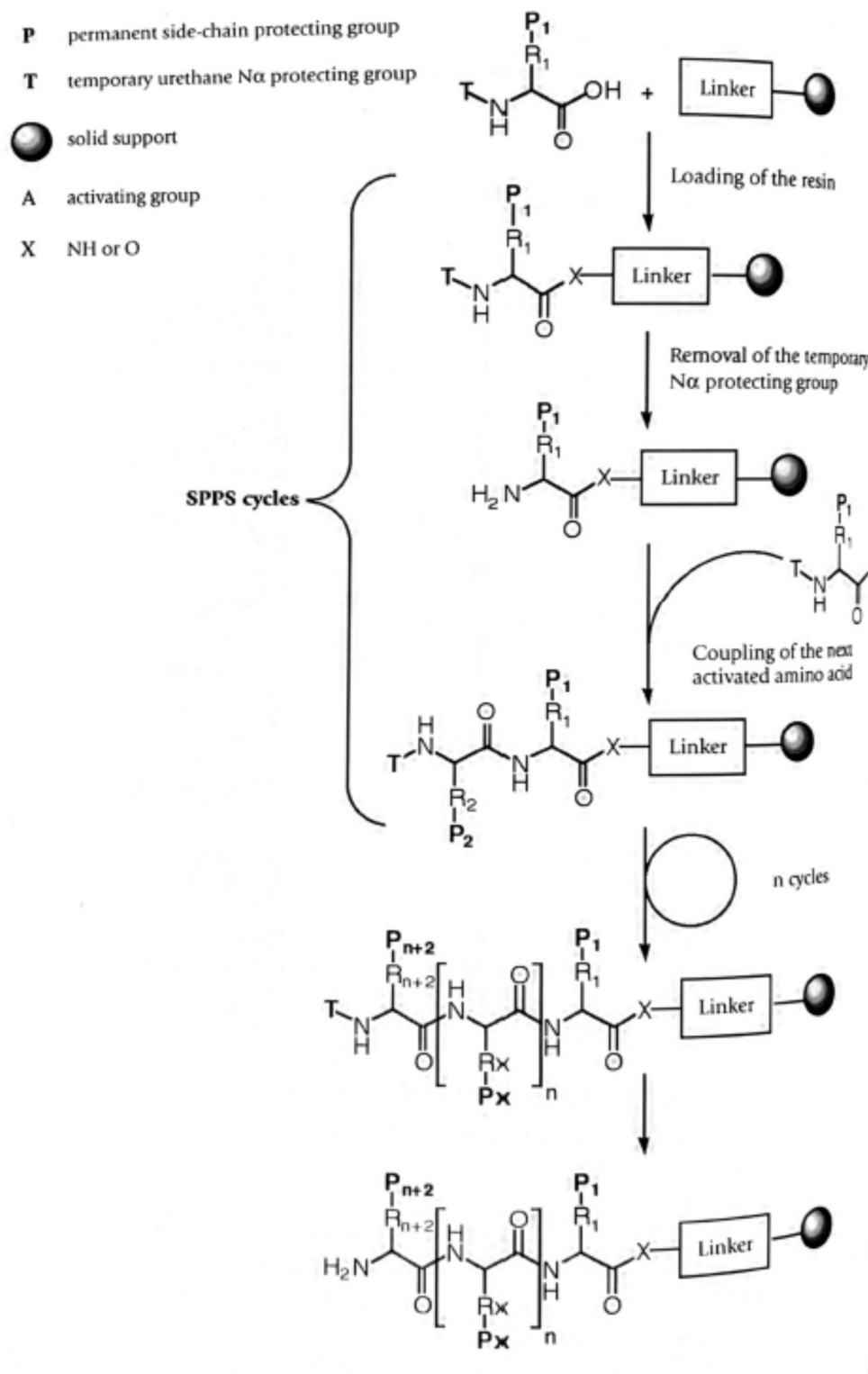
59. The amide forming reaction is straightforward. See Ex. 1059 (Kiso) at 10. It involves first “activating” a carboxyl group. This makes it simpler and more reactive to allow use of further mild reaction conditions. *Id.* at 10-11. The activated carboxyl group then reacts with an amino group to yield an amide bond:



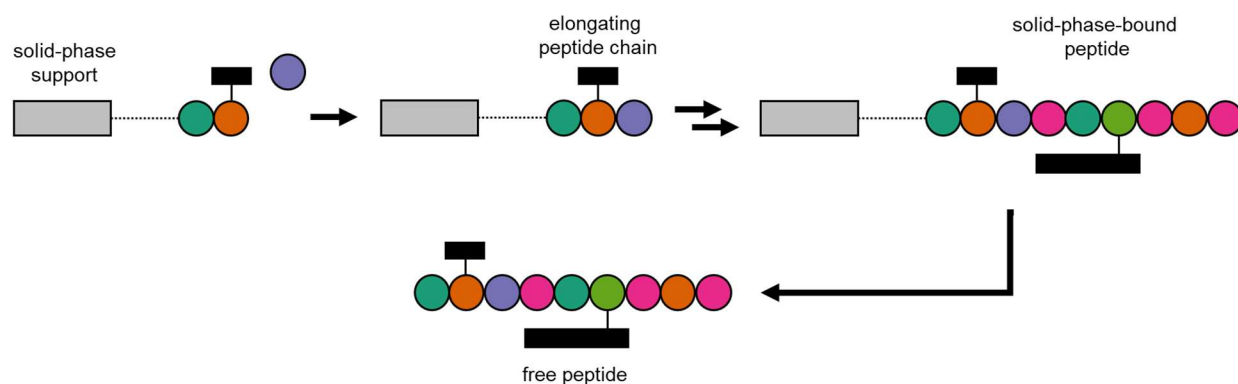
Id. at 11. Each amino acid bearing an N-amino protecting group is activated and coupled to the carboxyl-terminal amino acid of the growing peptide chain one by one. *Id.*

60. Solid-phase peptide synthesis is a common technique that offers many advantages over conventional solution synthesis because of its simplicity of operation, providing the ideal means for peptide and protein synthesis. The technique is called “solid phase” because it involves the peptide not being synthesized free in solution but rather tethered to an insoluble solid-phase resin,

which makes washing and purification simple. The basic procedure of solid-phase peptide synthesis is shown in the following figure:



Ex. 1058 (Amblard) at 10. Peptide synthesis entails a one-by-one stitching together of peptide monomer building blocks, reacting the nucleophilic amine group of one to an activated electrophilic carbonyl group of another. *See id.* at 10-11. The building blocks are commonly supplied as amino acids in “protected” form. *Id.* at 11. This means that the reactive amino group of an amino acid is protected by a base-labile group such as *N*-Fmoc, and any reactive side-chains of the amino acid are protected by an acid-labile group such as *t*-Bu. In this way, side reactions of monomers to each other or to side-chains can be avoided. Overall, the process involves iteratively chaining together single building blocks, usually on a solid support, followed by release of the free peptide without significant handling or purification steps like column chromatography. The following diagram depicts the concept:

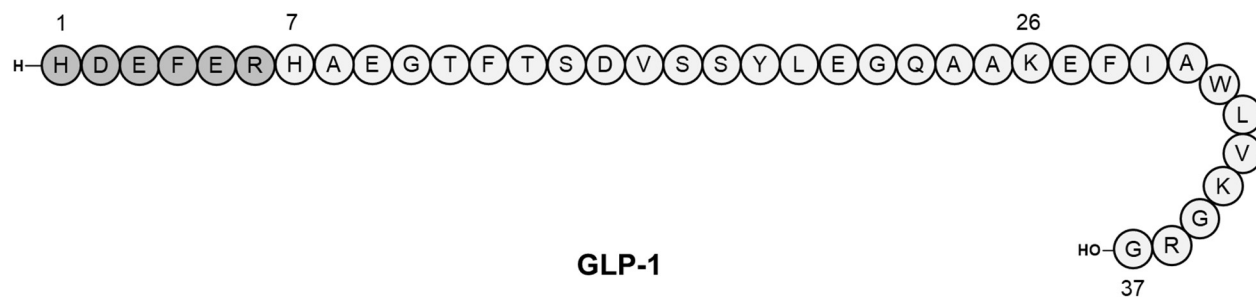


61. As a testament to the simplicity and repeatability of the process, solid-phase peptide synthesis has found numerous applications not only by peptide

chemists but also by biochemists, biologists, pharmacologists, physiologists, endocrinologists, and immunologists. *See, e.g.,* Ex. 1039 (Meienhoffer).

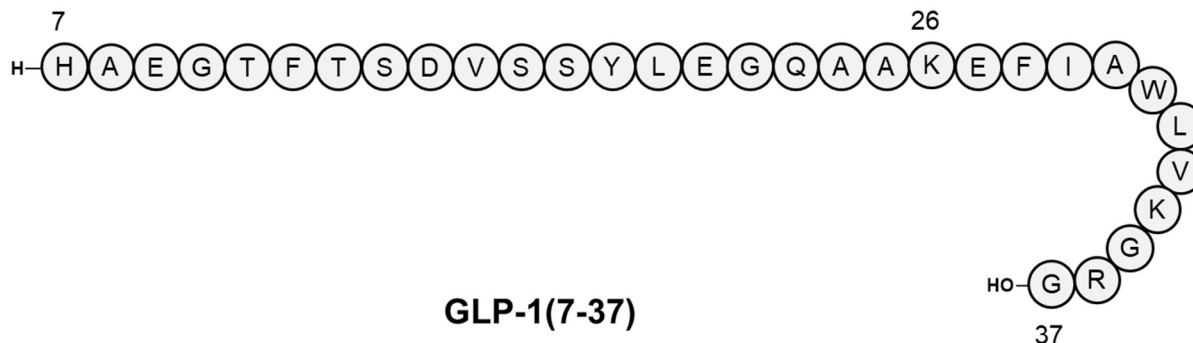
62. As of the priority date, conducting these reactions and synthesizing peptides had become very routine, and largely automated. Ex. 1039 (Meienhoffer) at 65-66. In practice, the POSA at the time would have had access to automated synthesizers, which were marketed by a variety of instrument manufacturers. Indeed, with peptide synthesizers it is particularly easy to make combinatorial libraries of peptides for screening, greatly enhancing the ease of performing high-throughput screening (e.g., the rapid synthesis and testing of numerous compounds).

63. Of particular focus here are two specific peptides: liraglutide and semaglutide, both of which are analogues of GLP-1(7-37), a naturally occurring peptide produced by the body. Native full-length, unprocessed GLP-1 (i.e., the peptide initially produced by the body) is a peptide containing 37 amino acids:

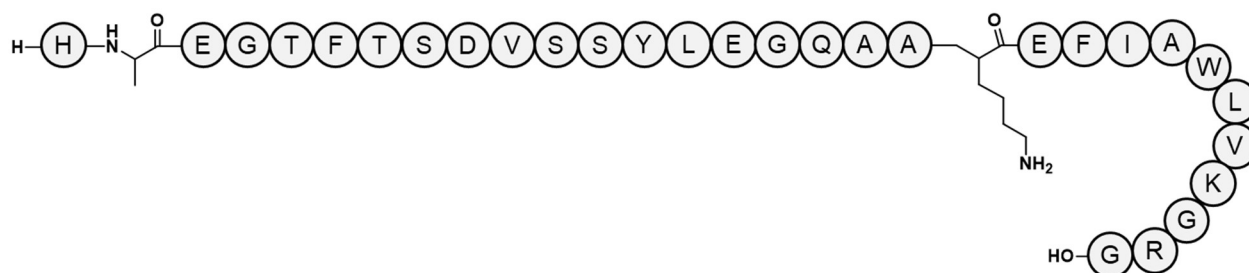


64. Native GLP-1(1-37) is then naturally converted into its truncated forms (i.e., GLP-1(7-37) and GLP-1(7-36)), with GLP-1(7-37) represented by the

following sequence that lacks the amino acids at positions 1-6 in unprocessed, full-length GLP-1:

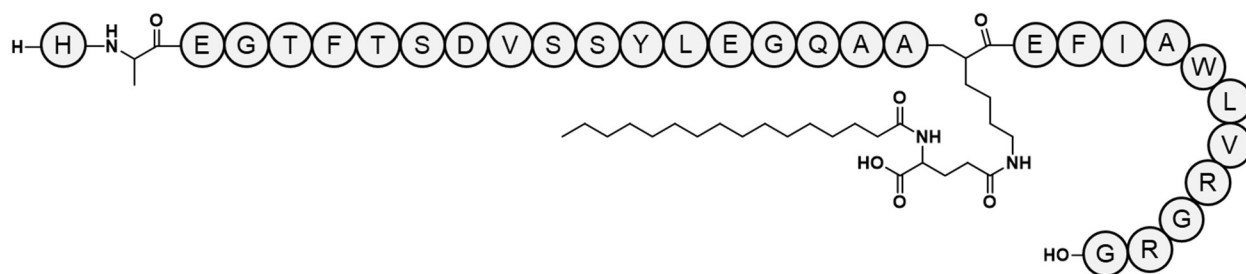


See, e.g., Ex. 1017 (Drucker 2001) at 4. Alternatively, GLP-1(7-37) can be depicted as follows:



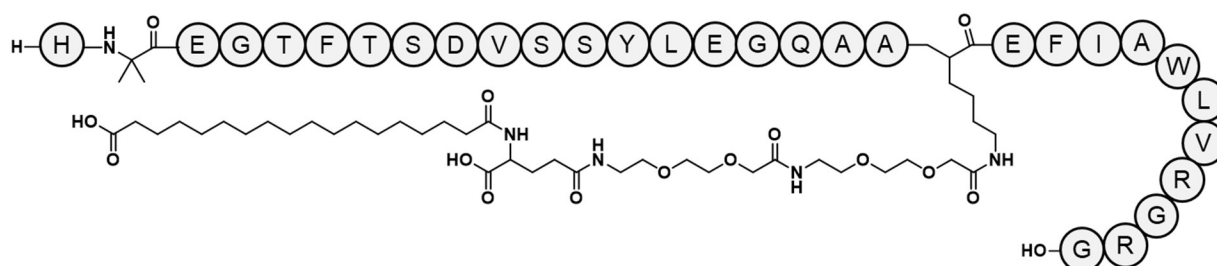
65. In the case of liraglutide and semaglutide, two primary differences exist as to the amino acid linkages versus the naturally occurring GLP-1(7-37). First, substitutions exist with respect to one (liraglutide) or two (semaglutide) amino acids. Second, the peptide is acylated through the connection of a fatty acid via a glutamic acid linker (liraglutide) or a combination of a glutamic acid linker and a di-AEEA spacer (semaglutide).

66. Liraglutide has the following structure:



See, e.g., Ex. 1031 (Holz) at 7. As can be seen above, liraglutide differs from naturally occurring GLP-1(7-37) in that it (1) contains an arginine (“Arg”) substitution at position 34 and (2) is acylated with a C₁₆ fatty acid via a glutamic acid spacer at position 26. Ex. 1031 (Holz) at 7; Ex. 1010 (Knudsen 2004) at 3; Ex. 1011 (Knudsen 2001) at 4-5.

67. Semaglutide has the following structure:



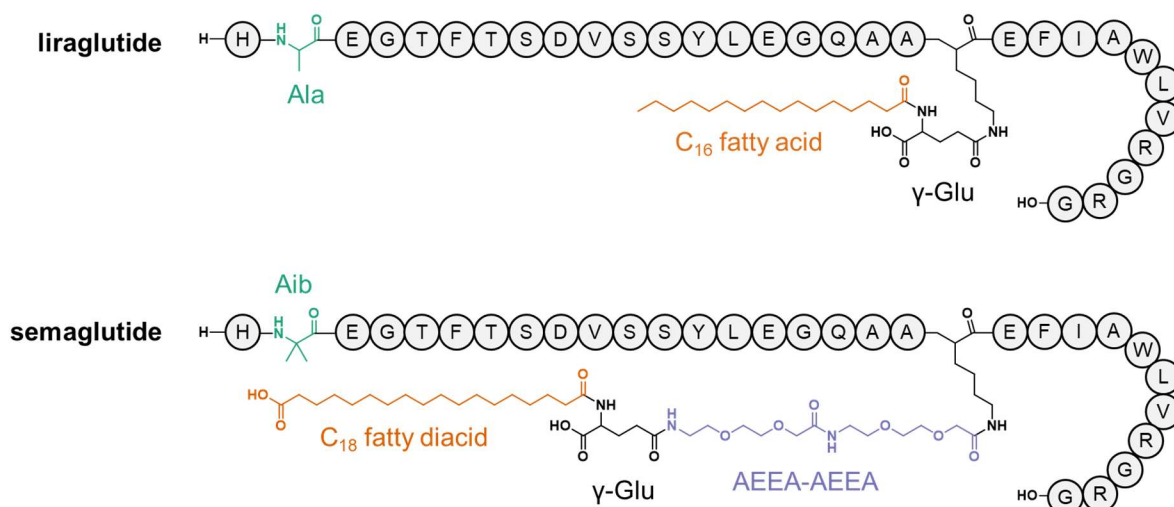
See Ex. 1063 (Ozempic Label) at Section 11. Semaglutide similarly differs from naturally occurring GLP-1(7-37) in that (1) it contains two amino acid substitutions: an Aib/Ala substitution at position 8 (i.e., “Aib⁸”) and, as with liraglutide, an Arg/Lys substitution at position 34 (i.e., “Arg³⁴”); and (2) the lysine

at position 26 is acylated with a C₁₈ fatty diacid via a glutamic acid linker, like liraglutide, and a di-AEEA⁴ spacer.

68. Liraglutide thus differs from naturally occurring GLP-1(7-37) in that there is an Arg³⁴ substitution and Lys²⁶ is acylated with a C₁₆ fatty acid via a glutamic acid linker. For semaglutide, there is the same Arg³⁴ substitution as in liraglutide, an additional Aib⁸ substitution, and Lys²⁶ is also acylated, but with a C₁₈ fatty diacid via a glutamic acid linker⁵ and a di-AEEA spacer. The following shows a comparison of liraglutide and semaglutide:

⁴ Synonyms used for this well-used chemical compound in the art include, among others, [2-(2-amino)ethoxy]ethoxy acetic acid (i.e., “AEEA”). Sato called di-AEEA “JJ.” Ex. 1045. Other references called AEEA “AdOO.” Ex. 1094 (Accardo). Others have called it “mini-PEG.” Ex. 1095 (Autio).

⁵ I note that both liraglutide and semaglutide use the (S)-isomer of glutamic acid (i.e., the naturally occurring L-form of glutamic acid, an amino acid).



69. Synthesizing both liraglutide and semaglutide would have been routine as of the priority date. With respect to semaglutide, the POSA would have chemically synthesized the adduct to the Lys²⁶ with any number of widely published techniques to specifically ensure reaction of the di-AEEA carboxylate with the ϵ -Lys amine. Orthogonal protection was a widely used method to ensure selective coupling to form the requisite amide bonds, not unlike typical peptide synthesis. *See, e.g.*, Ex. 1006 (Albericio). This routine method would have entailed the chemist choosing commercially available protected amino acids—which may include reactive amino or carboxy reactive groups on a side chain, like lysine and glutamic acid—where the additional amine or carboxylate would be protected differently (orthogonally) from the groups required for peptide synthesis. That strategy would avoid a side-reaction by allowing the specific deprotection of one protective group in a multiple-protected structure without affecting the others. These groups are protected from usually used chemicals encountered in peptide

synthesis and are removed in the final synthesis step, which typically includes the removal of the peptide from a polymeric resin. Additionally, as of the priority date, protected AEEA monomers were commercially available that could be used in solid-phase peptide synthesis applications. *See, e.g.*, Ex. 1016 (Corey 2002) at 2 (disclosing AEEA as being obtained from PE Biosystems in Foster City, CA); Ex. 1061 (EP '750) at [0046]-[0047] (disclosing “commercially available” Fmoc-protected 8-amino-3,6-dioxaoctanoic acid as brand-name PNA Expedite™ linker); Ex. 1064 ('676 patent) at 12:62-13:21 (similar).

70. It is also my opinion that the remaining structural aspects of semaglutide would have likewise been routine to prepare in view of the art.

71. For example, to make a peptide containing Aib⁸, the POSA would simply use commercially available, amino-protected Aib in place of alanine, using the identical automated process and procedure described above. Like any commonly used amino acid, Aib has long been commercially available in a protected form used in solid-phase synthesis. *See, e.g.*, Ex. 1066 ('743 publication) (filing date Sept. 9, 2004) at [0147], [0161] (using commercially obtained Fmoc-Aib-OH in manual solid-phase peptide synthesis); Ex. 1065 ('486 patent) (filing date July 12, 2000) at 12:3, 23:61-25:67 (obtaining Fmoc-protected amino acids commercially, and incorporating Aib into GLP-1 analogue by solid-phase peptide synthesis). A POSA would have known that the addition of amino acids to the

growing peptide chain occurs in a precise, step-wise and repetitive manner. While the common methods of peptide synthesis have some differences, they all follow the same step-wise method to add amino acids one-at-a-time to the growing peptide chain. The automated procedures are straightforward, and essentially driven by choices the chemist makes within a software program. *See, e.g.,* Ex. 1058 (Amblard) at 10. For example, the chemist would input instructions to have the synthesizer repeatedly perform reactions resulting in the deprotection and coupling of an amine to the carboxy group of the next amino acid to stitch together a designed sequence. *Id.*

72. To incorporate the di-AEEA spacer onto the existing glutamic acid spacer, the POSA would again make use of well-established coupling techniques of an amine to an acid, similar to peptide synthesis. As stated above, protected AEEA monomers were commercially available and common as of the priority date. *Supra* ¶ 69. This synthesis too could be accomplished via software and an automated process using an off-the-shelf peptide synthesizer, because, as stated earlier, the reactive functional amino and carboxy groups are identical to those in amino acids (e.g., glycine). Even if an automated process or machine were not used, however, manual solid-phase synthesis would have likewise been trivial because the process was extraordinarily well-known (having been around for

nearly half a century), and the materials necessary readily available. *See generally*, e.g., Ex. 1036 (Lehninger); Ex. 1067 (Sewald).

VIII. SCOPE AND CONTENT OF THE PRIOR ART

73. I identify below the prior art references supporting my expert opinions. All references cited throughout this declaration would have been known and publicly available to a POSA as of the priority date. While I have identified specific references herein, I am also relying on the skill, knowledge, and expertise of a POSA based on what was known at the time of invention, without referring to a specific document, including but not limited to the background and other general statements provided in this declaration.

74. The prior art disclosures identified in this declaration would have been understood by a POSA in light of the background knowledge that they would have had by the priority date of the '122 patent. To the extent I list or describe a reference below but do not rely on it as a ground for arguing the challenged claims are obvious, that prior art should be understood to form part of the background knowledge of a POSA, and to support the POSA's motivation to combine the prior art with a reasonable expectation of success. Further, to the extent I cite a reference for just one or several of its teachings, I reserve the right to rely on that reference for any other aspect of its teaching.

75. For every item of prior art discussed herein, I reserve the right to establish that the subject matter described therein has an earlier effective date or first publication date earlier than indicated.

1. The Knudsen Patent (Ex. 1012)

76. The Knudsen Patent (U.S. Patent No. 6,268,343) is titled “Derivatives of GLP-1 Analogues” and issued on July 31, 2001. I understand that the Knudsen Patent is prior art to the ’122 patent under at least 35 U.S.C. § 102(b) because it issued more than one year before the earliest priority date of the ’122 patent.

77. The Knudsen Patent explained that the GLP-1 analogues “preferably have only one or two [lysine residues] wherein the ϵ -amino group of one or both [lysine residues] is substituted with a lipophilic substituent.” Ex. 1012 (Knudsen Patent) at 12:23-26. The Knudsen Patent further disclosed that the purpose of the lipophilic substituent was “[t]o obtain a satisfactory protracted profile of action of the GLP-1 derivative.” *Id.* at 16:57-58. “The lipophilic substituent may be attached to an amino group of the GLP-1 moiety by means of a carboxyl group of the lipophilic substituent which forms an amide bond with an amino group of the amino acid residue to which it is attached.” *Id.* at 16:61-65. The lipophilic substituent could be an “acyl group of the formula $\text{HOOC}(\text{CH}_2)_m\text{CO—}$, wherein m is an integer from 4 to 38,” including “ $\text{HOOC}(\text{CH}_2)_{16}\text{CO—}$.” *Id.* at 19:17-22; *see*

also id. at 16:59-61 (“The lipophilic substituents preferably comprises 4–40 carbon atoms, in particular 8–25 carbon atoms.”).

78. The Knudsen Patent disclosed that the lipophilic substituent could be attached to the peptide using a “spacer,” *id.* at 17:55-57, and that several “suitable spacers” could be used. *Id.* at 17:59-60.

79. The Knudsen Patent also disclosed liraglutide as one such GLP-1 analogue and described its favorable properties. *See, e.g., id.* at claims 1, 14, 187:41-188:4. For example, liraglutide demonstrated a significantly improved protraction profile (i.e., half-life), *id.* at 192:30-60 (Table 1, Example 37), and reported efficacy data showing that liraglutide retained its antidiabetic properties. *Id.* at 193:35-45.

80. Another GLP-1 analogue disclosed and claimed by the Knudsen Patent is Arg³⁴Lys²⁶ (N^ε-(ω-carboxyheptadecanoyl))-GLP-1(7-37)-OH. *Id.* at 177:33-57, Claim 10. Like liraglutide, this GLP-1 analogue replaced the native lysine residue at position 34 with arginine. Also like liraglutide, this analogue was acylated at Lys²⁶, but this time with a C-18 fatty diacid rather than liraglutide’s C-16 fatty acid. The Knudsen Patent provided data that showed that this analogue exhibited a longer half-life than liraglutide. *Id.* at 192:30-60, Table 1 (*compare* Example 11, Arg³⁴Lys²⁶ (N^ε-(ω-carboxyheptadecanoyl))-GLP-1(7-37)-OH, *with* Example 37, liraglutide).

TABLE 1

Test com- pound*)	Hours after sc. Administration								
	0.75	1	2	4	6	8	10	12	24
GLP-1 (7-37)		100	9	1					
Exam- ple 25	73	92	100	98	82	24	16	16	16
Exam- ple 17	76	71	91	100	84	68	30		9
Exam- ple 43		39	71	93	100	91	59	50	17
Exam- ple 37		26	38	97	100	71	81	80	45
Exam- ple 11	24	47	59	71	100	94	100		94

2. Knudsen 2001 (Ex. 1011)

81. Knudsen 2001 is titled “GLP-1 Derivatives as Novel Compounds for the Treatment of Type 2 Diabetes: Selection of NN2211 for Clinical Development” and was published in 2001. I understand Knudsen 2001 is prior art to the ’122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the earliest priority date of the ’122 patent.

82. Knudsen 2001 disclosed GLP-1 analogues derivatized with long fatty acids, spacers, and/or amino acid substitutions. Ex.1011 (Knudsen 2001) at 1. According to Knudsen 2001,

Fatty acids or fatty diacids, optionally extended with a ‘spacer’ between the epsilon-amino group of the lysine side chain and the carboxyl group of the fatty acid, were used. Acylation with simple fatty acids increases the net negative charge of the resulting molecule with one (by blocking the epsilon-amino group of the lysine), whereas

peptides acylated with a L-glutamoyl-spacer or with diacids provides a further increase of the negative charge. The addition of a negative charge to the acylated molecule is expected to improve solubility at physiological pH.

Id. at 3.

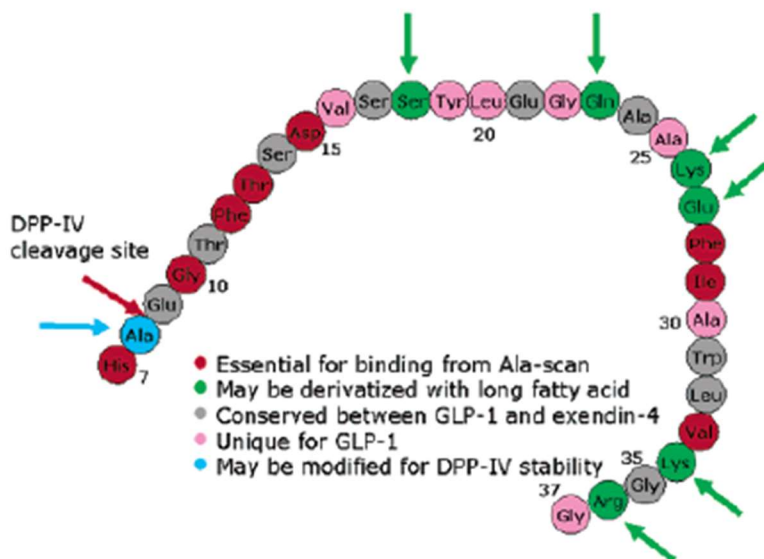
83. Knudsen 2001 concluded that liraglutide (i.e., γ -L-glutamoyl(N- α -hexadecanoyl)-Lys²⁶,Arg³⁴-GLP-1(7-37)) “was selected as the best compound” because the γ -L-Glu spacer “gave the most potent compound,” and because it was derivatized at the naturally occurring lysine residue at position 26. *Id.*.

3. Knudsen 2004 (Ex. 1010)

84. Knudsen 2004 is titled “Glucagon-like Peptide-1: The Basis of a New Class of Treatment for Type-2 Diabetes” and was published on July 20, 2004. I understand Knudsen 2004 is prior art to the ’122 patent under at least 35 U.S.C. § 102(a) because it was published before the earliest priority date of the ’122 patent. I have been informed that while Knudsen 2004 is § 102(a) prior art, it is possible that in the right circumstances Patent Owner could remove it as prior art. My opinions below remain the same, however, regardless of whether Knudsen 2004 is prior art. While it provides interesting teachings, it is not necessary for my analysis and ultimate opinions provided below.

85. Knudsen 2004 disclosed information about various GLP-1 analogues under investigation at the time, and basic information about the naturally occurring

GLP-1 peptide. *See generally*, Ex. 1010 (Knudsen 2004). First, detailed structure-activity-relationship (“SAR”) information for GLP-1(7-37) was disclosed:



Id. at 3. Second, Knudsen 2004 explained that Novo Nordisk prepared a series of acylated GLP-1 derivatives, including liraglutide, based on this information. *Id.* According to Knudsen 2004, Novo Nordisk sought to develop GLP-1 analogues “aimed at being long-acting via two independent mechanisms, self-association and noncovalent binding to plasma albumin fatty acid binding sites, resulting in a pharmacokinetic profile with slow absorption and a long half-life. Albumin thus serves as a buffer reservoir for liraglutide.” *Id.* Thus, Knudsen 2004 explained that GLP-1 analogues can be prepared using fatty acids and various spacers. *Id.*

4. Bridon (Ex. 1014)

86. Bridon (U.S. Patent No. 6,514,500) is titled “Long Lasting Synthetic Glucagon Like Peptide {GLP-1}” and issued on February 4, 2003. I understand

Bridon is prior art to the '122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the date of the application for patent in the United States.

87. Bridon disclosed “modified insulintropic peptides capable of forming a peptidase stabilized insulintropic peptide.” *See* Ex. 1014 (Bridon) at Abstract. More specifically, Bridon disclosed GLP-1 analogues modified with “chemical moieties that link or connect reactive groups to” extend the half-life of the peptide. *Id.* at 1:10-15. A “preferred linking group [was] AEEA ([2-(2-amino)ethoxy)]ethoxy acetic acid).” *Id.* at 3:19-20. Further, Bridon explained that certain criteria were used when determining where to modify the GLP-1 analogues: “if a carboxylic group, not critical for the retention of pharmacological activity is available on the original ITP and no other reactive functionality is present on the ITP, then the carboxylic acid is chosen as attachment point for the linker-reactive entity modification.” *Id.* at 16:46-52. Thus, Bridon disclosed GLP-1 analogues containing di-AEEA spacers attached to lysine residues, which contain a carboxylic acid side chain. *See, e.g., id.* at 2:4-13, 28:56-30:67 (Example 5), 31:45-32:22 (Example 7). The di-AEEA spacers were added to the GLP-1 peptides using automated peptide synthesizers. *Id.* at 14:10-13, 24:5-37.

5. Sato (Ex. 1045)

88. Sato is an International Patent Application Publication, titled “KDR and VEGF/KDR Binding Peptides and Their Use in Diagnosis and Therapy” and was published on September 12, 2003. I understand Sato is prior art to the ’122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the date of the application for patent in the United States.

89. Sato generally disclosed the use of a di-AEEA spacer to modify therapeutic peptides. *See, e.g.*, Ex. 1045 (Sato) at 22:14-17; *see also id.* at 115-17 (Example 4, Table 10), 124-25 (Experiment B), 145-46 (Table 11), 179-80 (Example 31), 189-90 (Example 37). Sato specifically disclosed N^ε-substitutions of lysine residues with a di-AEEA spacer and a lipophilic group (e.g., a dicarboxylic acid derivative). *See, e.g., id.* at Figures 55, 58, 62, 63.

6. Rando 1980 (Ex. 1043)

90. Rando 1980 is titled “Functional incorporation of synthetic glycolipids into cells,” and was published in 1980. I understand that Rando 1980 is prior art to the ’122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the earliest priority date of the ’122 patent.

91. Rando 1980 disclosed that varying the length of linkages between a sugar residue and cholesterol anchor in synthetic glycolipids could improve a compound’s properties, and highlighted that “[i]t is of interest to compare the

effects of the spacer group length on the susceptibility of the modified erythrocytes to aggregate.” Ex. 1043 (Rando 1980) at 3. Rando 1980 further disclosed that the authors “ha[d] shown that ... glycolipids with one or two spacer groups are incorporated to the same extent into the erythrocyte,” but that, for example, “[f]urther increases ... do not appear to enhance the aggregation response.” *Id.* at 3-4. Rando 1980 notably taught that the hydrophilic nature of PEG-based molecules made them particularly effective as a linker/spacer when used between hydrophobic molecules and compounds, and explained that with “hydrophobic ... spacer groups,” “[i]t could be anticipated that ... relatively large distances between [a] sugar residue and cholesterol anchor would have to be achieved before the sugar could functionally interact with a binding protein,” as “[t]he hydrophobic chains would [tend to] either ‘ball up’ and interact with themselves in the aqueous environment or they would dissolve in [a] lipid bilayer.” *Id.* at 1. But Rando 1980 disclosed that the *hydrophilic* nature of PEGs provided an answer, and that “[i]n order to circumvent this problem [they] ha[d] developed a hydrophilic spacer group that combines the water solubility of ethers with the ease of polymerization of amino acids,” and specifically that “3,6-dioxa-8-aminooctanoic acid [i.e., AEEA]-based spacer groups can be functionally incorporated into cells.” *Id.*

7. Kim (Ex. 1033)

92. Kim is titled “Development and Characterization of a Glucagon-Like Peptide 1-Albumin Conjugate” and was published in 2003. I understand that Kim is prior art to the ’122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the earliest priority date of the ’122 patent.

93. Kim disclosed information related to a GLP-1 analogue known as CJC-1131. *See* Ex. 1033 (Kim) at Abstract. Kim explained that CJC-1131 was a GLP-1(7-37) analogue having a D-alanine substitution at position 8 and a lysine residue at position 37 with a 2-[2-[2-maleimidopropionamido(ethoxy)ethoxy]] acetamide substituent (i.e., AEEA-maleimide). *Id.* at 3. CJC-1131 had the same half-life as albumin, about 19 days, because the AEEA-maleimide substituent enabled CJC-1131 to permanently bind to albumin *in vivo*. *Id.* at 4, 8. This reinforced that an AEEA-based spacer moiety could be used to attach an albumin-binding component (here, maleimide) to a GLP-1 analogue, even in combination with an N-terminal modification (here, a D-Ala⁸ substitution).

8. Markussen (Ex. 1038)

94. Markussen is titled “Soluble, Fatty Acid Acylated Insulins Bind to Albumin and Show Protracted Action in Pigs” and was published in 1996. I understand that Markussen is prior art to the ’122 patent under at least 35 U.S.C.

§ 102(b) because it was published more than one year before the earliest priority date of the '122 patent.

95. Markussen taught insulins acylated with fatty acids at the ϵ -amino group of a lysine residue. *See* Ex. 1038 (Markussen) at Abstract. Markussen explained that “[w]hen the [negative] charge is moved closer to the fatty acid substitution ... the affinity for albumin increases. Possibly, the charge mimics the carboxylate group of a fatty acid and thereby enhances binding to albumin.” *Id.* at 7. Markussen concluded that the “[m]odification of a peptide by fatty acids may be a general method” to improve the duration of action of a peptide. *Id.*

9. Peters (Ex. 1042)

96. Peters is titled “Ligand Binding by Albumin” and was published in 1995. I understand that Peters is prior art to the '122 patent under at least 35 U.S.C. § 102(b) because it was published more than one year before the earliest priority date of the '122 patent.

97. Peters disclosed general information about albumin. Ex. 1042 (Peters) at 23-33. For example, Peters explained that human-serum albumin is a protein containing 585 amino acid residues, *id.* at 27, 39, and that albumin can interact with long chain fatty acids (i.e., C₁₆-C₂₀), including fatty acids having an 18-carbon chain. *Id.* at 93 (long chain fatty acids “bind in a hydrophobic pocket that is adaptable to the ligand, with its negative charge matched in a salt bond by the

positive charge of a nearby lysyl or arginyl residue.” Among the long-chain fatty acids, stearic acid exhibited the strongest binding affinity for albumin. *Id.* at 99.

10. Additional prior art references and knowledge

98. In addition to the references discussed herein, I utilize the knowledge and expertise of a POSA. In this declaration, I also cite to references in addition to those recited and summarized above, including to provide background on the state of the art at the priority date. All references, whether summarized above or below, that were publicly available prior to the priority date of the '122 patent are within the scope of the prior art.

IX. THE POSA WOULD HAVE BEEN MOTIVATED TO USE A DI-AEEA SPACER AND C₁₈ FATTY DIACID WITH A REASONABLE EXPECTATION OF SUCCESS

99. As noted, the POSA would have known as of the priority date that liraglutide used a γ -L-glutamic acid (or glutamyl) linker (also equivalently called a spacer) to bind a fatty acid (on one end of the linker) to the lysine residue at position 26 of the peptide (at the other end of the linker). *Supra* Sections VII.B, VIII.A.1-2. The POSA would have understood that acylating a peptide with a fatty acid in this manner significantly increased its half-life, and that physically separating the fatty acid from the peptide with the glutamic acid linker improved a number of the peptide's properties, including receptor binding and potency. Given the POSA's motivation to develop an improved GLP-1 analogue with less frequent

administration, however, it would have been obvious to further modify this linkage, as well as the fatty acid, to improve peptide half-life even further relative to liraglutide. As explained above, I understand in addition to explaining that a POSA would have been motivated to modify the linker and fatty acid used in liraglutide, Dr. Flatt has also opined that a POSA would have been motivated to make an Aib⁸ substitution to liraglutide to impart protection from DPP-IV. In my opinion, a POSA would have recognized that pairing an N-terminal modification (i.e., an Aib⁸ substitution) with the spacer-fatty acid modifications at Lys²⁶ (using a di-AEEA spacer and C₁₈ fatty diacid) would provide two distinct mechanisms by which the analogue's half-life would be improved: DPP-IV protection via the N-terminal modification and stronger albumin binding via a modified spacer-fatty acid substituent at Lys²⁶. While I agree with Dr. Flatt, my opinions focus on specific aspects related to the spacer and fatty acid modifications.

100. Particularly, in my opinion, the POSA considering available prior art references such as Bridon in view of the POSA's knowledge of the state of the art, would have had ample motivation to further extend the linker component of liraglutide with the addition of a di-AEEA spacer, with a reasonable expectation that the resulting compound would successfully demonstrate improved half-life

and receptor binding (or at least half-life and receptor binding that would be superior to native GLP-1(7-37)).⁶

101. Moreover, given references such as the Knudsen Patent and Knudsen 2004, and the POSA's knowledge of the art, the POSA would also be motivated to modify the fatty acid used in liraglutide with a C₁₈ fatty diacid, with a reasonable expectation of success.

A. The POSA would have been motivated to modify liraglutide by adding a di-AEEA spacer to the existing glutamic acid linker with a reasonable expectation of success.

102. Acylation of peptides with fatty acids, including the use of linker or spacer molecules between the peptide and fatty acid such as was done with liraglutide, was a well understood technique to improve a peptide's properties as of the priority date.

⁶ Though I often refer to the existing glutamic acid moiety in liraglutide as a "linker," and the di-AEEA molecule in semaglutide as a "spacer," these terms are interchangeable, and the use of one over the other at any point does not reflect any substantive difference in my opinions. *See, e.g.*, Ex. 1010 (Knudsen 2004) at 3 (referring to the "γ-Glu amino acid [in liraglutide] as a *spacer*" (emphasis added)); Ex. 1001 ('122 patent) at 5:29-32 (defining "the term 'hydrophilic *linker*' as ... a *spacer* that separates a peptide and an albumin binding residue" (emphasis added)).

103. Indeed, publications had long-described the concept of connecting fatty acids to peptides to facilitate “albumin binding” and increase their duration of action, or half-life ($t_{1/2}$). *See, e.g.*, Ex. 1035 (Kurtzhals) at 1 (concluding “that the biochemical concept of albumin binding can be applied to protract the effect of insulin,” and thus “that derivatization with albumin-binding ligands could be generally applicable to prolong the action profile of peptide drugs”); Ex. 1010 (Knudsen 2004) at 3 (explaining that such “acylated derivatives of GLP-1 ... are aimed at being long-acting via two independent mechanisms, self-association and noncovalent binding to plasma albumin fatty acid binding sites, resulting in a pharmacokinetic profile with slow absorption and a long half-life”); Ex. 1015 (Clodfelter) (describing His⁷, Arg²⁶ GLP-1(7-37) derivatized with a C₈ fatty acid); Ex. 1007 (Baggio) at 1 (“[R]ecombinant or covalent coupling of smaller peptides to serum albumin represents an emerging strategy for extending the circulating $t_{1/2}$ of the target peptide.”). In contrast to peptides, which are cleared very quickly from circulation, albumin has a significantly longer half-life in the body. Albumin had also been shown to bind effectively to various fatty acids. Connecting one of those fatty acids to the peptide thus allows albumin to serve as a carrier for the peptide, significantly increasing half-life. In this manner, “[a]lbumin thus serves as a buffer reservoir for [peptides such as] liraglutide.” Ex. 1010 (Knudsen 2004) at 3.

Or, as Sato described it, “a chemical ‘warhead’ for therapy.” Ex. 1045 (Sato) at 55:15. This was a well-understood concept in therapeutic peptide development.

104. Additionally, the use of spacers or linking groups to physically separate bioactive peptides from albumin-binding moieties, such as the glutamic acid linker used in liraglutide, was also well-established and routine in peptide design by the priority date. Indeed, it was taught in textbooks. *See, e.g.*, Ex. 1067 (Sewald) at 3 (explaining that “[t]he synthesis of analogues of bioactive peptides with unusual amino acid building blocks, *linker or spacer molecules* and modified peptide bonds is directed towards the development of potent agonists and antagonists of endogenous peptides,” and that “[o]nce the amino acids of a protein that are essential for the specific biological mode of action have been revealed, these pharmacophoric groups may be incorporated into a small peptide” (emphasis added)); Ex. 1045 (Sato) at 55:3-7 (disclosing that the “introduction of linkers or spacers between the targeting sequence of the ... complex binding peptide and the detectable label or therapeutic agent ... may improve the relevant properties of the binding peptide (e.g., increase stability, etc.)”); Ex. 1019 (Ekwuribe) at 3:1-15 (recognizing preexisting “techniques of stabilizing peptide and protein drugs in which proteinaceous drug substances are conjugated with relatively low molecular weight compounds such as ... fatty acids” (citing R. Igarishi et al., “Proceed. Intern. Symp. Control. Rel. Bioact. Materials”, 17, 366, (1990); T. Taniguchi et al.

Ibid 19, 104, (1992); G. J. Russel-Jones, Ibid, 19, 102, (1992); M. Baudys et al., Ibid, 19, 210, (1992))).

105. To that end, the POSA would have recognized that linker or spacer groups between a peptide and fatty acid provide multiple benefits. One such benefit comes from the ability to reduce interference between the separate activities of fatty acid binding to albumin, on the one hand, and peptide binding to GLP-1 receptors, on the other. Because albumin is a much larger and bulkier protein than the GLP-1 peptide, it can get in the way and interfere with the amino acids on the peptide as they attempt to bind to GLP-1 receptors (also a large protein). With a spacer between the fatty acid and the peptide, however, the albumin protein is kept further away from the peptide backbone, so the peptide has more space to bind to the GLP-1 receptor (i.e., they are less likely to interfere with each other). This, in turn, leads to enhanced binding to GLP-1 receptors and increased potency of the peptide. *See, e.g.*, Ex. 1045 (Sato) at 55:3-7, 125:4-8; Ex. 1010 (Knudsen 2004) at 3 (“GLP-1 can be acylated at multiple sites with a hexadecanoyl fatty acid and a γ -Glu amino acid as a spacer, and the acylated GLP-1 exhibits retained potency and a long half-life after subcutaneous administration.”); Ex. 1017 (Drucker 2001) at 8 (“GLP-1 peptides acylated with simple fatty acids alone, or with a L-glutamoyl spacer or diacids exhibit an

enhanced negative charge, leading to greater albumin binding and improved solubility at physiologically relevant pH.”)

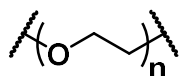
106. Given these well-understood principles, the POSA would have had a clear understanding of the role of the glutamic acid linker in liraglutide and the benefits it conferred. Further, a POSA would have been motivated to retain the same glutamic acid linker (i.e., the (S)-isomer, or L-form), which is the naturally occurring form of glutamic acid, and would not have considered using the (R)-isomer of γ -glutamic acid (i.e., the non-naturally occurring D-form). But a POSA that was motivated to improve upon liraglutide would not be required to, and would not, constrain itself to the use of only that same linker. Rather, the POSA would certainly recognize that further modification of the linkage between the fatty acid and peptide would be fertile ground to achieve even greater overall benefits. And given the benefits already conferred by the glutamic acid linker, the POSA would have had ample motivation to extend that connection with a PEG-based moiety (known to be highly effective as a spacer), and more specifically, to use one or more instances of the AEEA molecule (which had repeatedly been used successfully in this capacity, including in GLP-1 analogue design).

107. As noted, the POSA would recognize that the glutamic acid linker used in liraglutide had already demonstrated a certain degree of effectiveness. The art further taught that use of this spacer contributed to a compound with increased

efficacy. *See, e.g.*, Ex. 1011 (Knudsen 2001) at 5 (“A γ -L-Glu spacer was preferred because it gave the most potent compound.”); *see also* Ex. 1034 (Knudsen 2000) at 2 (“Acylation with simple fatty acids increases the net negative charge of the resulting molecule by 1, whereas peptides acylated with a L-glutamoyl spacer or with diacids provide a net increase of the negative charge by 2. In the two latter cases, an enhanced effect on binding to albumin is predicted. This extra negative charge added to the acylated molecule is also expected to provide a higher solubility at physiological pH.”). While the POSA would thus be motivated to retain that existing linker, which had shown to be effective with liraglutide, the POSA would also recognize that additional modifications to the overall linkage, such as extending its length, could provide even greater benefits (e.g., creating more space between albumin and the peptide backbone). *See, e.g.*, Ex. 1043 (Rando 1980) at 3-4 (teaching that varying the length of such linkages could lead to improved results, and noting, for example, that “[i]t is of interest to compare the effects of the spacer group length on the susceptibility of the modified erythrocytes to aggregate”); *id.* (“We have shown that ... glycolipids with one or two spacer groups are incorporated to the same extent into the erythrocyte,” while “[f]urther increases ... do not appear to enhance the aggregation response”); *see also, e.g.*, Ex. 1045 (Sato) at 29:10-13 & Fig. 77 (studying “binding of ... derivatives with different spacer length”); Ex. 1030 (Holst 2004) at 5 (“The apparent retention of

high biological activity in spite of the presence of the albumin moiety contrasts strikingly to [liraglutide], the intrinsic activity of which is clearly lowered by albumin binding (by ~2–3 orders of magnitude), suggesting that the site of attachment to albumin is of importance and that *the linker position and length in 2211 could be suboptimal.*” (emphasis added)).

108. To do so, it would have been a natural and obvious choice for the POSA to turn to proven, non-harmful polyethylene glycol (“PEG”)-based moieties to extend the linkage, and specifically, to use one or more instances of the PEG-based AEEA moiety used in semaglutide. PEG linkers are those that have the following substructure:



109. PEG linkers are frequently used in peptides or proteins because of their water solubility, lack of toxicity, and low immunogenicity. Indeed, studies regarding this process of “mask[ing] the protein surface by covalent coupling of soluble poly(ethylene glycol) (PEG) or, as it has become known, ‘pegylation,’” can be traced back to the 1950s.⁷ Ex. 1029 (Veronese) at 1-2. And “[t]he pioneering

⁷ “PEG, as obtained by ethylene oxide polymerization, presents one or two terminal hydroxyl groups (mPEG-OH or HO-PEG-OH) depending upon the initiator of polymerization: methanol or water, respectively. A branched form may also be ob-

first steps in pegylation [itself] were taken in [the] late 1970s.” *Id.* at 1. Notably, while “[o]ther soluble polymers, including polysaccharides and albumin, ha[d] been used for protein conjugation, primarily to stabilize proteins toward proteolysis and to increase residence time in the body,” the POSA would understand that “it was *the development of pegylation that provided the real breakthrough* in enhancing the pharmaceutical properties of proteins and peptides.” *Id.* at 2. Indeed, years before the priority date, it was “clear that pegylation [wa]s well on its way to becoming a standard component of the pharmaceutical tool box.” *Id.* at 1 (emphasis added).⁸

tained, as well as products with multiple reactive groups, at one or at both extremes,” with “the monofunctional polymers, linear or branched, [which] are used for protein modification.” Ex. 1041 (Pasut) at 2.

⁸ The use of PEGs for this purpose was recognized repeatedly throughout the prior art. *See, e.g.*, Ex. 1041 (Pasut) at 1 (problems including “short *in vivo* half-life (due to enzyme degradation or rapid kidney clearance) and physiochemical drawbacks, for example low solubility or instability ... immunogenicity and antigenicity” are “often addressed by covalent linking of the pharmaceutically active molecule to polymers, . . . *PEG being the most successful* among those used” (emphasis added)); *id.* at 2 (“The second generation of conjugates was an improvement on the first,” including with respect to “spacers between the polymer and the drug [that]

110. Notably, the unique properties of PEG-based moieties conferred several benefits that made them well-regarded for use as spacers, particularly in the context of the POSA's motivations here. As Veronese described, "PEG possesses a unique set of properties, including absence of toxicity, immunogenicity and antigenicity; low, mass-dependent elimination via the kidney; high flexibility and

were studied to allow the release of bound drug under specific triggering conditions."); Ex. 1029 (Veronese) at 3 (reporting in the context of antibodies with short circulation times, that "[t]he half-life problem can be solved by pegylation."); Ex. 1019 (Ekwuribe) at 6:41-47 (disclosing "covalently conjugated peptide complexes where-in the peptide is covalently bonded to one or more molecules of a polymer incorporating as an integral part thereof a hydrophilic moiety, e.g., a linear polyalkylene glycol, and wherein said polymer incorporates a lipophilic moiety as an integral part thereof"); Ex. 1014 (Bridon) at 2:4-13, 3:10-20, 28:56-30:67 (Example 5), 31:45-32:22 (Example 7); Ex. 1033 (Kim) at 3-4, 8; Ex. 1045 (Sato) at 73:11-12 (describing the use of "a spacer [which are] typically PEGs of different molecular weights"); Ex. 1012 (Knudsen Patent) at 17:55-60 (disclosing several "suitable spacers" for purposes of attaching a "lipophilic substituent ... to the ϵ -amino group of a [lysine] residue contained in the parent peptide"); Ex. 1043 (Rando 1980) (discussed below as using the PEG-based, AEEA molecule as such a spacer).

high solubility in water and certain organic media. In turn, PEG-proteins possess reduced toxicity, immunogenicity and antigenicity, reduced rates of kidney clearance and proteolysis, and enhanced solubility and stability. Valuable improvements in various pharmacokinetic kinetics parameters (e.g., absorption rate and volume of distribution are also seen.” Ex. 1029 (Veronese) at 2. Roberts similarly emphasized that that the “[r]easons for PEGylation (i.e. the covalent attachment of PEG) of peptides and proteins are numerous and include shielding of antigenic and immunogenic epitopes, shielding receptor-mediated uptake by the reticuloendothelial system (RES), and preventing recognition and degradation by proteolytic enzymes. PEG conjugation also increases the apparent size of the polypeptide, thus reducing the renal filtration and altering biodistribution. An important aspect of PEGylation is the incorporation of various PEG functional groups that are used to attach the PEG to the peptide or protein.” Ex. 1044 (Roberts) at Abstract.

111. The POSA would have recognized several of these benefits as particularly useful here. For instance, PEGs are hydrophilic (i.e., they improve water solubility because they have a strong tendency to bind water) due to an intrinsic capacity to hydrogen-bond with water molecules, which renders them particularly effective as a linker/spacer between a hydrophobic (low solubility in water) fatty acid and a hydrophobic peptide like GLP-1, enhancing the solubility of

the combined molecule in aqueous solutions critical for an injectable therapeutic. To this end, Rando 1980 recognized more than forty years ago that with “hydrophobic ... spacer groups,” “[i]t could be anticipated that ... relatively large distances between [a] sugar residue and cholesterol anchor would have to be achieved before the sugar could functionally interact with a binding protein,” as “[t]he hydrophobic chains would [tend to] either ‘ball up’ and interact with themselves in the aqueous environment or they would dissolve in [a] lipid bilayer.” Ex. 1043 (Rando 1980) at 1. This “balling up” or aggregation is problematic in that it would cause a compound to precipitate out of aqueous solution, resulting in lowered drug concentrations and thus reduced efficacy of a therapeutic. But Rando 1980 disclosed that the *hydrophilic* nature of PEGs provided an answer, explaining that “[i]n order to circumvent this problem [they] ha[d] developed a hydrophilic spacer group that combines the water solubility of ethers with the ease of polymerization of amino acids,” and specifically that “3,6-dioxa-8-aminooctanoic acid [i.e., AEEA]-based spacer groups can be functionally incorporated into cells.”

*Id.*⁹

⁹ See also, e.g., Ex. 1044 (Roberts) at 11 (“[H]eterobifunctional PEGs bearing appropriate functional groups may be used to link two entities where a hydrophilic, flexible, and biocompatible spacer is needed,” and “can be used in a variety of ways that includes linking macromolecules to surfaces (for immunoassays, biosen-

112. Rando 1980's observation follows from basic chemical principles: that hydrophobic compounds have a high likelihood of self-association (e.g., selectively interacting with itself) when located in an aqueous medium—such as a drug formulation—resulting in the compound precipitating out of solution. Thus, hydrophobic compounds, like GLP-1(7-37) and liraglutide, are difficult to work with in solution and to formulate (e.g., because they risked needle clogging when administered parenterally to patients). *See* Ex. 1034 (Knudsen 2000) at 1. Thus, it is important to ensure that the drug product is unlikely to precipitate out of solution at the low temperatures and/or at high concentrations that may be needed for formulation and administration purposes. A POSA would have known that one way to decrease that likelihood is by incorporating hydrophilic elements, such as PEG-based moieties like AEEA, into the compound.

113. Moreover, that PEGs are chemically, toxicologically, and immunogenically inert would have also made them particularly attractive for purposes of modifying liraglutide. As noted, liraglutide already included a glutamic acid linker that contributed certain properties to an existing chemical balance. The ability to extend that linkage with a molecule that would not otherwise affect that balance would be highly desirable. PEG-based moieties were

sors or various probe applications), targeting of drugs, liposomes and viruses to specific tissues, liquid phase peptide synthesis and many others.”).

well known for exactly those properties, and thus this would have further motivated the POSA to select them for use as a spacer here. *See, e.g.*, Ex. 1029 (Veronese) at 2 (noting that “PEG possesses a unique set of [favorable] properties, including absence of toxicity, immunogenicity and antigenicity,” and further reporting from studies involving “conjugation of PEG to two of the three amino groups of insulin” that “PEGs of low mass were shown ... not to alter the conformation or activity of the hormone, but they did eliminate immunogenicity, antigenicity and allergenicity and lengthen circulation lifetime”); Ex. 1019 (Ekwuribe) at 2:30-41 (describing one prior art publication—Abuchowski and Davis (“Soluble polymers-Enzyme Adducts”, In: Enzymes as Drugs, Eds. Holcenberg and Roberts, J. Wiley and Sons, New York, N.Y., (1981))—that “disclose[d] various ways of conjugating enzymes with polymeric materials. More specifically, ... polyethylene glycol,” in which “[t]he resulting conjugated polypeptides are reported to retain their biological activities and solubility in water,” and a prior art patent—U.S. Pat. No. 4,179,337—by the same authors that similarly “disclose[d] that polyethylene glycol rendered proteins soluble and non-immunogenic when coupled to such proteins.”); *id.* at 2:60-65 (describing yet another prior art publication—Boccu et al., in Pharmacological Research Communication 14, 11-120 (1982)—that “disclosed that polyethylene glycol could be linked to a protein such as super-oxide dismutase” in which “[t]he resulting

conjugated product showed increased stability against denaturation and enzymatic digestion”).¹⁰

114. In addition to the above general advantages that would have motivated a POSA to use a PEG-based spacer here, the state of the art would have specifically motivated the POSA to use one or more instances of the AEEA molecule. Indeed, Rando 1980 reported the successful use of an AEEA spacer more than forty years ago. As did multiple others in the ensuing years before the priority date, including in its multiple, di-AEEA configuration, and even more specifically for use in GLP-1 analogue design.

115. Bridon, particularly, taught the use of a di-AEEA spacer group for use in GLP-1 analogue design, and would have motivated a POSA to use such a spacer to modify liraglutide here. Bridon taught “chemical moieties that link or connect

¹⁰ *See also, e.g.,* Ex. 1045 (Sato) at 55:1-12 (teaching that, in addition to the general “introduction of linkers or spacers between the targeting sequence of the ... complex binding peptide and the detectable label of therapeutic agent ... [which] may improve the relevant properties of the binding peptide (e.g., increase serum stability, etc.),” that such “linkers may include ... polyethylene glycol [PEG] derivatives, [as well as] amino acid spacers,” and that “combinations of the [these] moieties ... can also be employed to confer special advantage to the properties of the peptide”).

reactive groups to [peptides],” and Bridon specifically taught the AEEA moiety as a “preferred linking group.” *See* Ex. 1014 (Bridon) at 3:10-20. Bridon further taught that two units of the preferred AEEA linking group (i.e., “di-AEEA”) could be used to increase the half-life of GLP-1 analogues via connection to a lysine residue, which contains the carboxylic acid group that Bridon explained was necessary for attachment. *See, e.g., id.* at Abstract, 16:46-52, 28:56-30:67 (Example 5), 31:45-32:22 (Example 7). And Bridon confirmed that these connections could easily be accomplished using automated peptide synthesis. *Id.* at 14:10-13, 24:5-37, 28:56-30:67 (Example 5), 31:45-32:22 (Example 7). In my opinion, these teachings would have directly motivated a POSA to pursue the use of a di-AEEA linking group in a liraglutide analogue—specifically, by using it to extend the existing connection between the GLP-1 backbone and the albumin-binding fatty-acid group at Lys²⁶, the only lysine residue present in liraglutide. And as explained below, a POSA would have been motivated to retain the glutamyl linker-fatty acid connection already present in liraglutide. The POSA would have further had a reasonable expectation of successfully synthesizing the peptide using automated techniques, and in improving solubility and achieving increased peptide half-life and efficacy benefits with the specific di-AEEA linker taught by Bridon and the state of the art regarding PEG-based spacer groups.

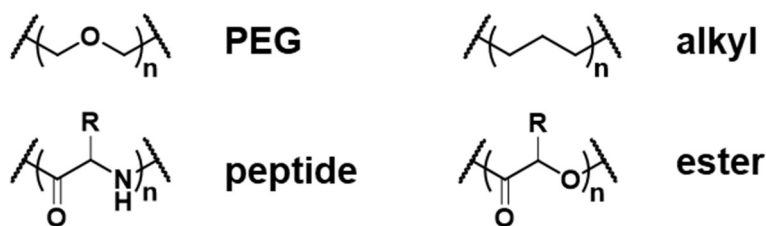
116. Bridon was not alone in disclosing the use of an AEEA (or di-AEEA) spacer, including in the context of GLP-1 analogue design. Sato, for instance, disclosed the use of spacers to “improve the relevant properties of the binding peptide,” (Ex. 1045 (Sato) at 55:6-7), and specifically disclosed the use of a di-AEEA spacer to modify certain peptides in this regard. *See, e.g.*, Ex. 1045 (Sato) at 22:14-17, 115-17 (Example 4, Table 10), 124-25 (Experiment B), 145-46 (Table 11), 179-80 (Example 31), 189-90 (Example 37). Indeed, Sato specifically disclosed N^ε-substitutions of lysine residues with a di-AEEA spacer and a lipophilic group (e.g., a dicarboxylic acid derivative). *See, e.g., id.* at Figures 55, 58, 62, 63. And Sato disclosed that its use of the di-AEEA spacer enhanced binding of certain peptides to their targets. *See, e.g., id.* at 125:4-8 (explaining that the di-AEEA spacer “can be helpful in enhancing binding to target molecule by multiple mechanisms. First, it could help reduce the steric hindrance between [the peptide and its target]. Second, it could provide extra length necessary to reach multiple binding sites available on a single cell.”); *see also id.* at 124:24-125:8, 179:1-180:28. Indeed, Sato emphasized that the “[a]ddition of a hydrophilic spacer between the peptide and the group used for attachment to the particle *should routinely be tested* with new targeting molecules as it improved the binding for both of the peptides evaluated [t]here.” *Id.* at 130:31-33 (emphasis added).

117. Kim similarly disclosed the use of an AEEA spacer in CJC-1131 to extend the half-life of the peptide. Ex. 1033 (Kim) at 3. Kim also confirmed that an AEEA spacer is unlikely to negatively impact albumin binding, particularly in situations in which (like liraglutide and semaglutide) a long fatty acid provides even more space than the small maleimide moiety used in CJC-1131.

118. Finally, the POSA would have further been motivated to use a di-AEEA spacer along with the existing glutamate to modify liraglutide in light of its polar nature and ability to facilitate hydrogen bonding. The POSA would have recognized that while the di-AEEA spacer could contribute to making the GLP-1 analogue more hydrophilic (as discussed above), using it along with the glutamate (instead of *replacing* the glutamate) would maintain the existing (and beneficial) net negative charge contributed by the glutamic acid linker and fatty acid. These features together would collectively contribute to making the more hydrophilic analogue easier to dissolve in water and thus less susceptible to formulation and manufacturing issues, with the net negative charge having the potential to further enhance albumin binding. *See, e.g.*, Ex. 1043 (Rando) at 4 (describing AEEA as a “hydrophilic spacer group”); Ex. 1034 (Knudsen 2000) at 2 (disclosing that the “extra negative charge added to the acylated molecule is also expected to provide a higher solubility at physiological pH”); Ex. 1038 (Markussen) at 7 (“Possibly, the charge mimics the carboxylate group of a fatty acid and thereby enhances binding

to albumin. When the fatty acid is substituted in position Phe^{B1} there is no negative charge in the vicinity, and the binding constant becomes lower by a factor of 4.”); Ex. 1034 (Knudsen 2000) at 2 (disclosing that “[a]cylation with simple fatty acids increases the net negative charge of the resulting molecule by 1, whereas peptides acylated with a L-glutamoyl spacer or with diacids provide a net increase of the negative charge by 2. In the two latter cases, an enhanced effect on binding to albumin is predicted”).

119. In addition to favoring PEG for the reasons explained above, a POSA would have been motivated to choose PEG over other general categories of linkers. There are a few general categories of linkers that are common for use in biological molecules. One alternative would be an alkyl linker (such as a fatty acid), but a fatty acid lacks the oxygen molecules of PEG and would imbue the molecule with greater hydrophobicity. Other alternatives, such as a peptide- or ester-based spacer would not necessarily suffer from the same hydrophobicity problem, but either of those linkages would be particularly prone to hydrolysis. PEG would have thus been a clearly preferred choice, from an already narrow field of candidate spacers:



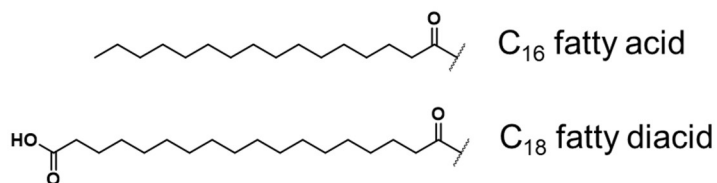
120. Among PEGs, the only meaningful variable would be the length of the PEG linker (i.e., number of repeated AEEA subunits). Although a POSA would be motivated by Bridon to use two AEEA (i.e., di-AEEA), a POSA would also screen 1, 2, 3, and 4 AEEA units, regardless. I also note that using 1, 2, 3, or 4 AEEA units would fall within the scope of claim 1 of the patent.

121. Finally, I understand that another of Mylan's experts, Dr. Dalby, has opined that a POSA would have been motivated to modify liraglutide by using a di-AEEA spacer connected to the fatty acid (via the existing glutamic acid linker) for additional reasons. Because a POSA would collaborate with others like Dr. Dalby, the motivations he identifies provide additional motivations to pursue this specific modification. While I understand that Dr. Dalby's declaration provides reasons for his opinions, I understand that he is generally offering the opinion that a formulator would have been motivated to implement a di-AEEA spacer in this manner because they would have expected that the di-AEEA spacer would have the benefit of making the GLP-1 analogue more hydrophilic, which a POSA would have expected to improve the solubility of the peptide while maintaining its ease of formulation and manufacture. Thus, for these additional reasons, a POSA would have been further motivated to modify liraglutide with a di-AEEA spacer, and would have reasonably expected it to result in a GLP-1 analogue that is easier to formulate.

122. Thus, particularly in view of the POSA's skill and knowledge of the state of the art, and together with Bridon and the additional disclosures discussed above, the POSA would have had ample motivation to modify liraglutide by extending its existing glutamic acid linker at Lys²⁶ with a di-AEEA spacer, and would have reasonably expected the resulting peptide to have a good half-life with improved solubility and desirable antidiabetic properties. The POSA's expectation in this regard would have been further supported by the fact that, as of the priority date, synthesis of the modified compound would have been trivial and a matter of routine skill to complete quickly as of the priority date. *See, e.g.*, Section VII.B, above. This would have likewise further motivated the POSA to use these routine techniques to implement known solutions, with an expectation of achieving predictable (and favorable) results.

B. The POSA would have been motivated to modify liraglutide by acylating a C₁₈ fatty diacid onto the di-AEEA spacer via a glutamic acid linker with a reasonable expectation of success.

123. As noted, semaglutide uses a C₁₈ dicarboxylic acid at Lys²⁶ (or "C₁₈ fatty diacid") rather than the C₁₆ monocarboxylic acid (or "C₁₆ fatty acid") used in liraglutide at Lys²⁶. *See* Section VII.B, above.



Particularly when considering the Knudsen Patent, and a POSA's knowledge of the state of the art, in my opinion a POSA would have been motivated to acylate a C₁₈ diacid onto the di-AEEA spacer via a glutamic acid linker with a reasonable expectation that the resulting peptide would have a longer half-life and desirable antidiabetic properties.

124. The Knudsen Patent disclosed liraglutide as one of a series of disclosed GLP-1 analogues that “preferably have only one or two [lysine residues] wherein the ϵ -amino group of one or both [lysine residues] is substituted with a lipophilic substituent.” *See* Ex. 1012 (Knudsen Patent) at Abstract, 12:23-26, 187:41-188:4. The Knudsen Patent further disclosed “a more preferred embodiment, [in which] there is only one” lysine residue in the peptide chain of the GLP-1 analogue, and that the lipophilic substituent was used “[t]o obtain a satisfactory protracted profile of action of the GLP-1 derivative.” *Id.* at 12:27-29, 16:57-58; *see also id.* at 16:65-67 (disclosing that “most preferably,” there is only “one lipophilic substituent.”).

125. Further, the Knudsen Patent instructed that the lipophilic group could be an “acyl group of the formula $\text{HOOC}(\text{CH}_2)_m\text{CO—}$, wherein m is an integer from 4 to 38,” including “ $\text{HOOC}(\text{CH}_2)_{16}\text{CO—}$ ” (i.e., a C₁₈ dicarboxylic acid), and could be attached to an amino group via an acylation reaction at a carboxyl group of the lipophilic substituent. *Id.* at 19:17-22, 16:61-65. “In a most preferred

embodiment, the lipophilic substituent is attached ... to the ε-amino group of a [lysine] residue contained in the parent peptide,” and this is “optionally via a spacer.” *Id.* at 17:55-57.

126. Among the other GLP-1 analogues disclosed in the Knudsen Patent were, for example, Arg³⁴Lys²⁶ (N^ε-(ω-carboxyheptadecanoyl))-GLP-1(7-37)-OH, which included an arginine substitution at position 34 (a lysine residue in native GLP-1(7-37)) and was acylated with a C₁₈ fatty diacid at the lysine residue at position 26 (i.e., Lys²⁶). *Id.* at 177:33-57, claim 10. Notably, this analogue’s half-life was longer than that of liraglutide. *See, e.g., id.* at 192:30-60, Table 1 (*compare* Example 11, Arg³⁴Lys²⁶ (N^ε-(ω-carboxyheptadecanoyl))-GLP-1(7-37)-OH, *with* Example 37, liraglutide).

127. The Knudsen Patent thus disclosed that a C₁₈ fatty diacid used at Lys²⁶ of a GLP-1 analogue resulted in a peptide with a longer half-life than liraglutide. And Knudsen 2004 confirmed that liraglutide and other GLP-1 analogues could be prepared using fatty acids and spacers so that the peptide would have a longer half-life thanks to its ability to bind to albumin. Ex. 1010 (Knudsen 2004) at 3. Given the POSA’s motivation to make a GLP-1 analogue having a longer half-life than liraglutide, an obvious modification would be to choose a fatty acid substituent having stronger binding with albumin than the C₁₆ monoacid used in liraglutide.

128. Accordingly, a POSA would have been motivated to modify liraglutide by acylating a C₁₈ fatty diacid to Lys²⁶ via a di-AEEA spacer and a γ -L-glutamic acid linker. Further, because the Knudsen Patent taught that a C₁₈ diacid results in a longer half-life, a POSA would have reasonably expected that including a di-AEEA spacer and glutamic acid linker would result in an even longer half-life. A POSA's knowledge of the state of the art would have further strengthened the motivation to make this modification.

129. The POSA would have been aware that C₁₈ fatty acids had stronger binding affinities for albumin than C₁₆ fatty acids. And the mechanisms by which fatty acids interacted with albumin were well-characterized. *See generally* Ex. 1008 (Cistola I); Ex. 1009 (Cistola II). Specifically, positively charged amino acid residue side chains in albumin interacted with negatively charged carboxyl groups on the fatty acids. Ex. 1008 (Cistola I) at 7; Ex. 1009 (Cistola II) at 5. Further, albumin and fatty acids interacted via non-covalent hydrophobic forces. Ex. 1008 (Cistola I) at 7; Ex. 1009 (Cistola II) at 5. Cistola I even used ¹³C NMR to show the order of filling and relative occupation of albumin's fatty acid binding sites. Ex. 1008 (Cistola I) at 8. Both Cistola references reported that the carboxyl group of a C₁₈ acid interacted with albumin at several high-affinity binding sites. Ex. 1008 (Cistola I) at Abstract; Ex. 1009 (Cistola II) at 5.

130. Kenyon also disclosed that the carboxylate group of fatty acids interact with amino acid residues in human serum albumin (“HSA”). Ex. 1032 (Kenyon) at Abstract, 3-4.

131. Further, Peters disclosed the structure of the albumin protein and its ability to interact with compounds. Ex. 1042 (Peters) at 23-33. Albumin can interact with numerous ligands, including long chain C₁₆-C₂₀ fatty acids, which “bind in a hydrophobic pocket that is adaptable to the ligand, with its negative charge matched in a salt bond by the positive charge of a nearby lysyl or arginyl residue.” *Id.* at 93; *see also id.* at 90-93. Peters also disclosed that stearic acid (a C₁₈ acid) exhibited stronger binding affinities for albumin than other fatty acids did. *Id.* at 99.

132. Tonsgard 1988 described the binding of fatty acids to albumin in equilibrium dialysis experiments. Tonsgard explained that “[t]he albumin molecule is composed of three nonidentical cylindrical domains, each of which has a narrow hydrophobic channel able to accommodate only one or two hydrocarbon chains. The ends of each domain form nonidentical sub-domains containing positively charged amino acid side chains.” Ex. 1040 (Tonsgard 1988) at 5. Tonsgard further disclosed that previous studies had “indicate[d] that medium-chain monocarboxylic fatty acids bind almost exclusively via hydrophobic interactions, whereas the binding of long-chain monocarboxylic acids is dependent on both

electrostatic and hydrophobic interactions. Three of the albumin subdomains are primary binding sites for long-chain monocarboxylic fatty acids. The other three subdomains are primary binding sites for medium- and short-chain fatty acids and drugs. Drugs bind along the rim of the subdomains through electrostatic interactions with positively charged amino acid groups.” *Id.* at 5-6.

133. By crystallographic analysis, Bhattacharya noted that “[t]he two principal drug binding sites, in sub-domains IIA and IIIA, are observed to be occupied by fatty acids and one of them (in IIIA) appears to coincide with a high-affinity long-chain fatty acid binding site.” Ex. 1060 (Bhattacharya) at 1. Bhattacharya further reported “it ha[d] been shown that at several binding sites the carboxylate head-group of the bound fatty acid is more rigidly anchored than the methylene tail.” *Id.* at 2. Experimental results demonstrated that “[a]t most sites ... the shape of the [electron] density, which had a significant broadening at one end, clearly indicated the position of the carboxylate moiety and thus defined the orientation of the bound lipid,” and that “[t]his usually placed the carboxylate group of the fatty acid adjacent to an amino acid side-chain with which it could make a salt-bridge or hydrogen bond.” *Id.* at 2. Specifically, at Site 4 which is longer and narrower, as the chain of the fatty acid increases the carboxylate head-group remains fixed and the methylene tail protrudes further into the hydrophobic tunnel. *Id.* at 6. At Site 5, as with Site 4, the largest fatty acid used (C18:0) was

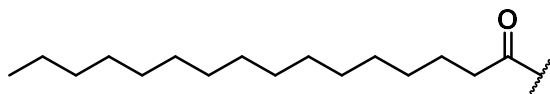
long enough to fill the channel completely. *Id.* The methylene tail of the C₁₈ acid traverses the entire width of the sub domain. *Id.* Results suggested that “only fatty acid species with at least 18 carbons atoms may span the distance between the polar ends of the pocket.” *Id.* Binding occurs “whether the pocket is short and wide (sites 1 and 3) or long and narrow (sites 2, 4, and 5),” which “allows both electrostatic and hydrophobic interactions to contribute to the binding energy and readily accounts for the progressive increase in binding affinity as the methylene tail lengthens.” *Id.* at 9. Bhattacharya additionally noted that their structural observations “are consistent with NMR data, which showed that binding sites with no significant electrostatic interaction between the protein and fatty acid are low-affinity sites.” *Id.*

134. Additionally, although the Knudsen Patent disclosed that various fatty acids would work at this position, the realistic list of candidates would have in fact been quite small. As explained above, the binding pocket for fatty acids with respect to albumin is about 18 carbons long. Accordingly, a POSA would have understood that fatty acids shorter than this would not take full advantage of all the possible binding surface, and a fatty acid longer would be unnecessarily larger than the binding pocket, thereby introducing potential complications without any increased benefit. Additionally, the shape of the binding pocket would mean that steric interactions would discourage the use of fatty acids with bends in them, such

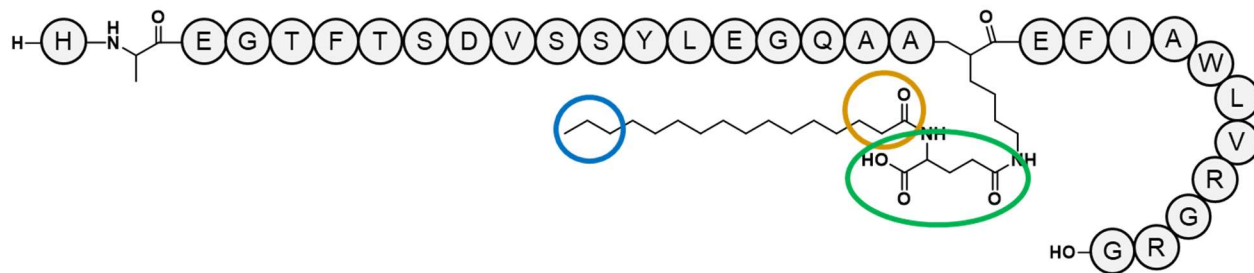
as unsaturated fatty acids (i.e., those with an alkene) or branched-chain fatty acids. Accordingly, the optimal length of the fatty acid chain to effectively bind to albumin would be a known result-effective variable, and one with a relatively small number of options to try.

135. In my opinion, a POSA would have been motivated, in view of the Knudsen Patent, to modify liraglutide by replacing the C₁₆ monoacid at Lys²⁶ with a C₁₈ dicarboxylic acid at Lys²⁶ with a reasonable expectation of success. In addition, a POSA would have reasonably expected this modification to result in a GLP-1 analogue having a longer half-life than liraglutide, or longer than GLP-1(7-37). This is reinforced by the state of the art and general understanding of a POSA as of the priority date of the '122 patent. For instance, as explained, a POSA would have understood the specific non-covalent interactions by which long-chain fatty acids bind to albumin. Those interactions would have led a POSA to a C₁₈ diacid for a few reasons.

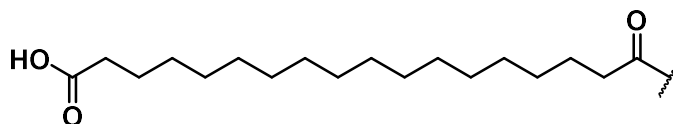
136. First, liraglutide used a C₁₆ *monoacid*, but that moiety had no free negatively charged carboxylate once it was incorporated into liraglutide:



137. This is illustrated in the following depiction of liraglutide:



138. Using a long-chain fatty *diacid*, however, would leave an extra carboxylic acid group that could interact with the positively charged residues on albumin. For example, the C₁₈ diacid disclosed in the art discussed above has the following structure once bound:



Incorporating this diacid would ensure there was a carboxylic acid group to interact with albumin, which a POSA would expect to have superior albumin binding (particularly for a C₁₈ fatty acid). *See, e.g.*, Ex. 1042 (Peters) at 99.

139. Finally, as discussed in Section IX.A, above, a POSA would have been motivated to retain the glutamic acid linker in liraglutide (i.e., γ -L-Glu). *See, e.g.*, Ex. 1034 (Knudsen 2000) at 2. As further noted, the POSA would have recognized that the art taught that the addition of a carboxylate group near a fatty acid on a peptide could enhance albumin binding. *See, e.g.*, Ex. 1038 (Markussen) at 7 (“Possibly, the charge mimics the carboxylate group of a fatty acid and thereby enhances binding to albumin. When the fatty acid is substituted in position Phe^{B1} there is no negative charge in the vicinity, and the binding constant becomes lower by a factor of 4.”). This is further confirmed in the figure depicted in paragraph 137, above (shown in green).

140. Fatty acid derivatives of proteins and peptides are well known in biological systems, and thus there exist natural processes to metabolize these moieties—avoiding both an immune response as well as possible toxic build-up (*see* Ex. 1062 (Schmidt) 1988 review). Additionally, fatty acids are largely inert and can tolerate most chemical reactions typically experienced in the course of peptide synthesis and purification.

141. Further, it would have been trivial to synthesize the C₁₈ diacid derivative. The C₁₈ diacid (i.e., octadecanedioic acid) would have been available and readily converted to a starting material such as an activated or protected ester that could be used in the same synthetic pathway used to make liraglutide, such as the synthetic pathways discussed in the Knudsen Patent. *See generally* Ex. 1012, Example 1 (preparing analogues using activated 2,5-dioxopyrrolidin-1-yl esters by standard acylation chemistry); Ex. 1096 ('532 patent) at 17:43-18:41, 23:5-20 (describing preparation of derivatives of C₁₈ diacid for acylation uses). The availability of the C₁₈ diacid as a starting material, and the ability to substitute it into an existing synthetic scheme (or use very trivial standard acylation chemistry) would have motivated a POSA to select it over acyl chains that were not as common or commercially available.

142. Finally, I understand Dr. Flatt has set forth additional reasons why a POSA would have been motivated to acylate a C₁₈ diacid to the di-AEEA spacer at Lys²⁶ via a glutamic acid linker. For those reasons in addition to those expressed herein, a POSA would have been further motivated to modify liraglutide in this way with a reasonable expectation of success. As noted above, the POSA's expectation in this regard would have been further supported by the fact that, as of the priority date, synthesis of the modified compound would have been trivial and a matter of routine skill to complete quickly as of the priority date. *See* Section VII,

above. This would have likewise further motivated the POSA to use these routine techniques to implement known solutions, with an expectation of achieving predictable (and favorable) results. Finally, a POSA would have at least considered using a C₁₈ diacid to be predictable given the known binding function of that fatty acid to albumin. And because it was known that there was a relationship between the length and charge of a fatty acid and binding to albumin, selection of the exact length and charge would have been a matter of simply optimizing those variables.

X. A POSA WOULD HAVE ARRIVED AT THE CLAIMED INVENTION THROUGH ROUTINE EXPERIMENTATION OF KNOWN VARIABLES

143. In my opinion, a POSA would have also been motivated to synthesize numerous liraglutide-based analogues to further improve its half-life, and would have therefore arrived at semaglutide through routine experimentation of testing known options for known variables or by optimization of known result-effective variables. While the design goal for liraglutide had been to make an analogue with a half-life longer than native GLP-1 (i.e., to go from minutes to hours), it would be incumbent on a POSA to make modifications to increase the circulating half-life to enable longer-lasting analogues. Thus, the liraglutide analogue could be modified by incremental changes to ensure, for example, once-weekly or twice-weekly efficacy without straying far from the known chemical and biological properties of the first-generation drug. Alternatively, a POSA would be motivated to seek other

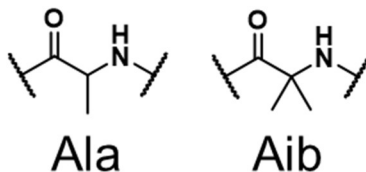
analogues to liraglutide that could also be administered less frequently and whose chemical properties were only trivially different for several reasons. Those analogues could be easier to produce, for instance, or cheaper, or less subject to patent protection, among other benefits.

144. Specifically, as I discussed previously, I understand Dr. Flatt has opined that a POSA would have been motivated to select liraglutide as a lead compound. Accordingly, in my opinion, a POSA would have been motivated to optimize the liraglutide compound by testing certain modifications to liraglutide.

145. As part of those modifications, a POSA would have understood that the structure-activity relationship of GLP-1 (and, by extension, liraglutide) was well-known. *See, e.g.*, Ex. 1005 (Adelhorst) at 1 (“side chains in positions 7, 10, 12, 13, and 15 are directly involved in the receptor interaction while positions 28 and 29 are important for GLP-1 to adapt the conformation recognized by the receptor”); Ex. 1010 (Knudsen 2004) at 3 (disclosing detailed SAR information); Ex. 1011 (Knudsen 2001) at 4-5 (disclosing that certain modifications were known to be effective while others negatively impacted the peptide). Specifically, a POSA would have known that two modifications to GLP-1 were beneficial and well tolerated: (1) substituting position 8 to protect it from cleavage by DPP-IV and (2) adding a lipophilic group to improve the half-life of GLP-1. A POSA would

also understand solubilizing a hydrophobic peptide like GLP-1 or liraglutide to be beneficial.

146. To protect position 8 from DPP-IV cleavage, a POSA would have understood that there were only a handful of options from which to choose. For instance, Dong disclosed a few “unnatural amino acids” that would protect position 8 from interactions with DPP-IV. Ex. 1013 (Dong) at 6. While there may have been others, the options were nonetheless limited, and a POSA’s general desire to protect position 8 from DPP-IV cleavage would have guided the testing of those limited options. In my opinion, this is nothing more than experimenting with known result-effective options (replacements for Ala⁸) to control a known result (protecting position 8 from cleaving with minimal impact on bioactivity). Among the known options, one obvious choice that the POSA would have reasonably expected to be successful would have been an Aib⁸ substitution, as taught by Dong. Indeed, while the POSA would have expected other position 8 modifications to have also been successful, Aib was both taught by the prior art and is structurally close to identical to the native Ala residue, and so would have been attractive as a very conservative modification that would be expected to have less negative impact on bioactivity than other modifications:



147. As for the lipophilic group, a POSA would have understood that liraglutide was already acylated at Lys²⁶ with palmitoyl (C₁₆ monoacid) and a glutamic acid linker to produce a compound with a superior half-life over GLP-1, but a POSA would have been motivated to test a number of other known options of lipophilic substituents and spacers to determine whether any may prove better than those used in liraglutide (or at the very least, acceptable). *See, e.g.*, Ex. 1011 (Knudsen 2001) at 3; Ex. 1010 (Knudsen 2004) at 3; Ex. 1012 (Knudsen Patent) at 12:23-26, 16:57-67. Moreover, a POSA would have understood that the size of the lipophilic substituent was constrained to avoid impacting the efficacy of the GLP-1-like analogue. *See, e.g.*, Ex. 1012 (Knudsen Patent) at 16:59-61 (“The lipophilic substituents preferably comprises 4–40 carbon atoms, in particular 8–25 carbon atoms.”). Additionally, as explained in paragraphs 132-134, above, a POSA would expect that the realistic set of useful fatty acids would be a small set of saturated, unbranched fatty acids (i.e., linear chains and avoiding alkenes) centered around 18 carbons in length. The set of fatty acids would also include monoacids or diacids, with the presence of carboxylic acids known to improve binding to albumin’s positively charged residues. *See, e.g.*, paragraphs 135-138, above. The length of

that fatty acid and the absence/presence of the second carboxylic acid would therefore be known result-effective variables with respect to albumin binding (and therefore half-life).

148. A spacer also serves a very well understood purpose: to link, but create space between, two compounds of interest—here, the lipophilic substituent and the GLP-1-like compound. Moreover, there would have been just a handful of well-known options to serve as spacers, one of which was AEEA (or di-AEEA). As explained in paragraphs 108-119, above, a POSA would have narrowed the field to uncleavable, inert, hydrophilic linkers like PEGs over the other common families of linkers like alkyl groups, amides, and esters. The exact structure of the PEG linker would not be expected to have much influence (analogous to how either a red or a blue bungee cord would work for the same purpose). However, AEEA would be a logical choice because it would have been an easily procured and used building block that had already been taught by the prior art as successful in analogous applications. *Id.* The selection of any one spacer (or even utilizing more than one known spacer), is not of particular importance beyond how the selection of the combined configuration with the compound being linked affects the properties-of-interest (e.g., half-life, efficacy) that are predominantly attributable to the linked compounds themselves. In sum, the length of the linker would be the predominant, key variable, which was a known result-effective

variable that affected solubility and half-life. *See id.* A POSA would have constrained screening efforts to a relatively small set, including 1, 2, 3, or 4 AEEAs.

149. Taken together, a POSA would have had a motivation to experiment throughout the limited options of lipophilic substituents and spacers to identify which contributed to a compound with the most desirable pharmaceutical properties for further development. Because the options were finite, a POSA would have again found this to be nothing more than routine experimenting with known options (lipophilic substituents and spacers) to control a known variable (improving the half-life compared to GLP-1). Among those known options would have been to acylate the Lys²⁶ position—like liraglutide—but to instead add a C₁₈ fatty di-acid and a di-AEEA spacer to the glutamic acid linker.

150. Moreover, a POSA would have found it straightforward to synthesize numerous analogues of liraglutide with these types of modifications. As of the priority date of the '122 patent, peptide synthesis had become so routine that it was effectively automated. Thus, simple push-button machines were capable of quickly synthesizing relatively complex peptides. Using such a machine to synthesize numerous derivatives of liraglutide as part of an initial screening procedure would have been routine and straight forward, and would have allowed a POSA to simply generate numerous derivatives and test their intended properties. In my opinion,

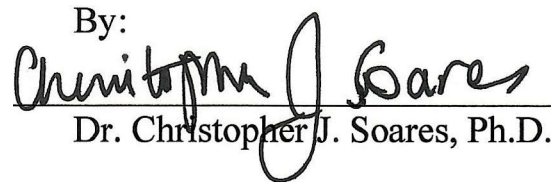
such a process would have led a POSA to synthesize and test semaglutide as one of several obvious options.

XI. CONCLUSION

151. For the reasons above, it is my opinion that the POSA would have had ample motivation to modify liraglutide by acylating Lys²⁶ with a di-AEEA spacer attached to the existing glutamic acid linker, and would have had a reasonable expectation that the modified peptide would demonstrate predictable results of a longer half-life with desired antidiabetic properties. In my opinion, a POSA would also be motivated to acylate the peptide with a C₁₈ fatty diacid rather than the C₁₆ monocarboxylic acid as used in liraglutide, with a reasonable expectation that the resulting peptide would demonstrate enhanced albumin binding. And, finally, it is also my opinion that a POSA's motivation to experiment with liraglutide as a lead to develop a compound with improved half-life and desirable antidiabetic properties would have arrived at semaglutide through routine experimentation of testing known options for known variables.

I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: 03/14/2023

By:

Dr. Christopher J. Soares, Ph.D.

APPENDIX A

APPENDIX A

Exhibit No.	Description
1001	U.S. Patent No. 8,536,122
1002	U.S. Patent No. 8,129,343
1003	Prosecution history excerpts for U.S. Patent No. 8,536,122
1004	Prosecution history excerpts for U.S. Patent No. 8,129,343
1005	Adelhorst, <i>Structure-Activity Studies of Glucagon-like Peptide-1*</i> , 269(9) J. BIO. CHEM. 6275 (1994)
1006	Albericio, <i>Orthogonal Protecting Groups for N(alpha)-amino and C-terminal carboxyl functions in Solid-Phase Peptide Synthesis</i> , 55(2) BIOPOLYMERS 123 (2000)
1007	Baggio, <i>A Recombinant Human Glucagon-Like Peptide (GLP)-1–Albumin Protein (Albugon) Mimics Peptidergic Activation of GLP-1 Receptor–Dependent Pathways Coupled With Satiety, Gastrointestinal Motility, and Glucose Homeostasis</i> , 53(9) DIABETES 2492 (2004)
1008	Cistola, <i>Carbon 13 NMR Studies of Saturated Fatty Acids Bound to Bovine Serum Albumin. I. The filling of Individual Fatty Acid Binding Sites</i> , 262(23) J BIOL CHEM. 10971 (1987)
1009	Cistola, <i>Carbon 13 NMR Studies of Saturated Fatty Acids Bound to Bovine Serum Albumin. II. Electrostatic Interactions in Individual Fatty Acid Binding Sites</i> , 262(23) J BIOL CHEM. 10980 (1987)
1010	Knudsen, <i>Glucagon-Like Peptide-1: The Basis of a New Class of Treatment for Type 2 Diabetes</i> , 47(17) J MED CHEM. 4128 (2004)
1011	Knudsen, <i>GLP-1 Derivatives as Novel Compounds for the Treatment of Type 2 Diabetes: Selection of NN2211 for Clinical Development</i> , 26(7) DRUGS OF THE FUTURE (2001)

Exhibit No.	Description
1012	U.S. Patent No. 6,268,343
1013	Dong, <i>Glucagon-Like Peptide-1 Analogs with Significantly Improved in vivo Activity</i> , in PEPTIDES: THE WAVE OF THE FUTURE (Michal Lebl et al. eds., 2001)
1014	U.S. Patent No. 6,514,500
1015	Clodfelter, <i>Effects of Non-Covalent Self-Association on the Subcutaneous Absorption of a Therapeutic Peptide</i> , 15(2) PHARM RSCH. 254 (1998)
1016	Nulf, <i>DNA Assembly using Bis-Peptide Nucleic Acids (bisPNAs)</i> , 30(13) NUCLEIC ACIDS RES.2782 (2002)
1018	Drucker, <i>Discovery, Characterization, and Clinical Development of the Glucagon-Like Peptides</i> , 127(12) J. CLIN. INVEST. 4217 (2017)
1019	U.S. Patent No. 5,359,030
1028	U.S. Patent No. 7,576,050
1029	Veronese, <i>Introduction and Overview of Peptide and Protein Pegylation</i> , 54 ADVANCED DRUG DELIVERY REVIEWS 453 (2002)
1030	Holst, <i>The Incretin Approach for Diabetes Treatment Modulation of Islet Hormone Release by GLP-1 Agonism</i> , 53 (suppl. 3) DIABETES S197 (2004)
1031	Holz, <i>Glucagon-Like Peptide-1 Synthetic Analogs: New Therapeutic Agents for Use in Treatment of Diabetes Mellitus</i> , 10 CURRENT MED. CHEM. 2471 (2003)
1032	Kenyon, <i>¹³C NMR Studies of the Binding of Medium-Chain Fatty Acids to Human Serum Albumin</i> , 35(3) J LIPID RES. 458 (1994)

Exhibit No.	Description
1033	Kim, <i>Development and Characterization of a Glucagon-Like Peptide 1-Albumin Conjugate: The Ability to Activate the Glucagon-Like Peptide 1 Receptor In Vivo</i> , 52(3) DIABETES 751 (2003)
1034	Knudsen, <i>Potent Derivatives of Glucagon-Like Peptide-1 with Pharmacokinetic Properties Suitable for Once Daily Administration</i> , 43(9) J MED CHEM. 1664 (2000)
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