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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

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Title of the Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS		
Attorney Docket Number	79532.8003.US03	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	3	**Suggested Figure for Publication (if any)**	

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Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Division of	13417137	2012-03-09

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
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Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13417137	non provisional of	61542100	2011-09-30
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13417137	non provisional of	61564668	2011-11-29
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( ) Person to whom the inventor is obligated to assign.	( ) Person who shows sufficient proprietary interest	

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Signature	/Patrick D. Morris/			Date (YYYY-MM-DD)	2013-02-22
First Name	Patrick D.	Last Name	Morris	Registration Number	53351

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METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

RELATED APPLICATIONS

[0001] The present application is a divisional of U.S. Patent Application No. 13/417,137, filed March 9, 2012 and now pending, which claims the benefit of U.S. Provisional Application No. 61/564,668, filed November 29, 2011, and U.S. Provisional Application No. 61/542,100, filed September 30, 2011, the disclosures of which are incorporated by reference herein in their entirety, including drawings.

BACKGROUND

[0002] Nitrogen retention disorders associated with elevated ammonia levels include urea cycle disorders (UCDs) and hepatic encephalopathy (HE).

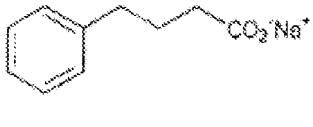
[0003] UCDs include several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia, including enzymes involved in the urea cycle. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. With reference to Figure 1, N-acetyl glutamine synthetase (NAGS)-derived *N*-acetylglutamate binds to carbamyl phosphate synthetase (CPS), which activates CPS and results in the conversion of ammonia and bicarbonate to carbamyl phosphate. In turn, carbamyl phosphate reacts with ornithine to produce citrulline in a reaction mediated by ornithine transcarbamylase (OTC). A second molecule of waste nitrogen is incorporated into the urea cycle in the next reaction, mediated by arginosuccinate synthetase (ASS), in which citrulline is condensed with aspartic acid to form argininosuccinic acid. Argininosuccinic acid is cleaved by argininosuccinic lyase (ASL) to produce arginine and fumarate. In the final reaction of the urea cycle, arginase (ARG) cleaves arginine to produce ornithine and urea. Of the two atoms of nitrogen incorporated into urea, one originates from free ammonia (NH_4^+) and the other from aspartate. UCD individuals born with no meaningful residual urea synthetic capacity typically present in the first few days of life (neonatal presentation). Individuals with residual function typically present later in childhood or even in adulthood, and symptoms may be precipitated by increased dietary protein or physiological stress *(*e.g., intercurrent illness).

[0004] Hepatic encephalopathy (HE) refers to a spectrum of neurologic signs and symptoms believed to result from hyperammonemia, which frequently occur in subjects with cirrhosis or

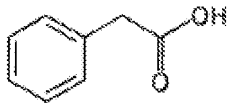
certain other types of liver disease. Subjects with HE typically show altered mental status ranging from subtle changes to coma, features similar to subjects with UCDs.

[0005] Subjects with nitrogen retention disorders whose ammonia levels and/or symptoms are not adequately controlled by dietary restriction of protein and/or dietary supplements are generally treated with nitrogen scavenging agents such as sodium phenylbutyrate (NaPBA, approved in the United States as BUPHENYL® and in Europe as AMMONAPS®) or sodium benzoate. These are often referred to as alternate pathway drugs because they provide the body with an alternate pathway to urea for excretion of waste nitrogen (Brusilow 1980; Brusilow 1991). NaPBA is a phenylacetic acid (PAA) prodrug. Another nitrogen scavenging drug currently in development for the treatment of nitrogen retention disorders is glyceryl tri-[4-phenylbutyrate](HPN-100), which is described in U.S. Patent No. 5,968,979. HPN-100, which is commonly referred to as GT4P or glycerol PBA, is a prodrug of PBA and a pre-prodrug of PAA.

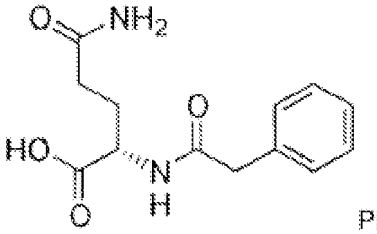
[0006] HPN-100 and NaPBA share the same general mechanism of action: PBA is converted to PAA via beta oxidation, and PAA is conjugated enzymatically with glutamine to form phenylacetylglutamine (PAGN), which is excreted in the urine. The structures of PBA, PAA, and PAGN are set forth below.



phenylbutyrate



Phenylacetic acid



Phenylacetylglutamine

[0007] The clinical benefit of NaPBA and HPN-100 with regard to nitrogen retention disorders derives from the ability of PAGN to effectively replace urea as a vehicle for waste nitrogen excretion and/or to reduce the need for urea synthesis (Brusilow 1991; Brusilow 1993). Because

each glutamine contains two molecules of nitrogen, the body rids itself of two waste nitrogen atoms for every molecule of PAGN excreted in the urine. Therefore, two equivalents of nitrogen are removed for each mole of PAA converted to PAGN. PAGN represents the predominant terminal metabolite, and one that is stoichiometrically related to waste nitrogen removal, a measure of efficacy in the case of nitrogen retention states. The difference between HPN-100 and NaPBA with respect to metabolism is that HPN-100 is a triglyceride and requires digestion, presumably by pancreatic lipases, to release PBA (McGuire 2010).

[0008] In contrast to NaPBA or HPN-100, sodium benzoate acts when benzoic acid is combined enzymatically with glycine to form hippuric acid. For each molecule of hippuric acid excreted in the urine, the body rids itself of one waste nitrogen atom.

[0009] Methods of determining an effective dosage of PAA prodrugs such as NaPBA or HPN-100 for a subject in need of treatment for a nitrogen retention disorder are described in WO09/1134460 and WO10/025303. Daily ammonia levels, however, may vary greatly in a subject. This can lead to overestimation by the physician of the average daily ammonia levels, which may result in overtreatment. Thus, there is a need in the art for improved methods for PAA prodrug dose determination and adjustment based on ammonia levels in subjects with nitrogen retention disorders such as UCDs or HE.

SUMMARY

[0010] Provided herein in certain embodiments are methods for determining whether to increase a dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder by measuring a fasting blood ammonia level and comparing the fasting blood ammonia level to the upper limit of normal (ULN) for blood ammonia, where a fasting blood ammonia level that is greater than half the ULN for blood ammonia indicates that the dosage needs to be increased. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35 μmol/L or 59 μg/mL. In certain embodiments, the methods include an additional step of administering an increased dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments administration of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments wherein a determination is made to administer an increased dosage of nitrogen scavenging drug

and wherein the nitrogen scavenging drug is a PAA prodrug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%.

[0011] Provided herein in certain embodiments are methods for determining whether to administer a nitrogen scavenging drug to a subject with a nitrogen retention disorder by measuring a fasting blood ammonia level and comparing the fasting blood ammonia level to the ULN for blood ammonia, where a fasting blood ammonia level that is greater than half the ULN for blood ammonia indicates that the nitrogen scavenging drug needs to be administered. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35 μmol/L or 59 μg/mL. In certain embodiments, the methods include an additional step of administering a nitrogen scavenging drug if the need exists, and in certain of these embodiments administration of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments wherein a determination is made to administer a nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA prodrug, the methods further include a step of determining an effective initial dosage of the PAA prodrug by determining a target urinary PAGN output based on a target nitrogen output and calculating an effective initial dosage that results in the target urinary PAGN output based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain embodiments, the methods include a step of administering the calculated effective initial dosage.

[0012] Provided herein in certain embodiments are methods for treating a nitrogen retention disorder in a subject who has previously been administered a nitrogen scavenging drug by measuring a fasting blood ammonia level, comparing the fasting blood ammonia level to the ULN for blood ammonia, and administering an increased dosage of the nitrogen scavenging drug if the fasting ammonia level is greater than half the ULN for blood ammonia. In certain embodiments, administration of an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject. In certain embodiments, the nitrogen retention disorder is a UCD or HE. In certain embodiments, the nitrogen scavenging drug is HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA). In certain embodiments, the ULN is around 35

μmol/L or 59 μg/mL. In certain embodiments wherein the nitrogen scavenging drug is a PAA prodrug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain embodiments, the methods include a step of administering the calculated effective dosage.

BRIEF DESCRIPTION OF DRAWINGS

[0013] Figure 1: The urea cycle and how certain nitrogen-scavenging drugs may assist in elimination of excessive ammonia.

[0014] Figure 2: Relationship between fasting ammonia and average ammonia UCD patients.

[0015] Figure 3: Venous blood ammonia values over 24 hours in (A) adult and (B) pediatric UCD patients.

DETAILED DESCRIPTION

[0016] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

[0017] In subjects with a nitrogen retention disorder, the desired effect of treatment with a nitrogen scavenging drug is control of blood ammonia level. Control of blood ammonia level generally refers to ammonia values within the normal range and avoidance of hyperammonemic crises, which are often defined in the art as transient ammonia values exceeding 100 μmol/L or 178 μg/mL accompanied by clinical signs and symptoms of hyperammonemia. Dosing of nitrogen scavenging drugs is usually based upon clinical assessment and measurement of ammonia. However, assessment of treatment effect and interpretation of ammonia levels is confounded by the fact that individual ammonia values vary several-fold over the course of a day and are impacted by timing of the blood draw in relation to the last meal and dose of drug (see, e.g., Lee 2010; Lichter-Konecki 2011; Diaz 2011).

[0018] A random ammonia value obtained during an outpatient visit may fail to provide a reliable measure of a subject's status and the drug effect. For example, basing treatment on a blood sample taken after eating a meal might overestimate average daily ammonia level and

result in overtreatment. Conversely, basing treatment on a blood sample taken after drug administration might underestimate average daily ammonia level and result in undertreatment. A fasting ammonia level at or near the ULN might be taken as an indication of satisfactory control without appreciating the fact that the ammonia burden during the day (average and/or highest possible value) might be significantly higher. Thus, a fasting level at or near the ULN may actually reflect undertreatment in a subject already a receiving nitrogen scavenging drug or the need for treatment in a subject not currently prescribed a nitrogen scavenging drug. A more accurate view of daily ammonia level could be obtained by multiple blood draws in a controlled setting over an extended period of time. Although this is currently done in clinical trials, it is clinically impractical.

[0019] As set forth below, the relationship between fasting ammonia levels and daily ammonia exposure was evaluated in subjects with nitrogen retention disorders. It was found that fasting ammonia correlates strongly with daily ammonia exposure, assessed as a 24 hour area under the curve for ammonia, daily average, or maximal daily concentration, and that a target fasting value which does not exceed half of the ULN is a clinically useful and practical predictor of ammonia values over 24 hours. As such, provided herein are clinically practical methods of evaluating ammonia exposure in subjects with nitrogen retention disorders based on fasting ammonia levels, as well as methods of using the resultant information to adjust the dosage of a nitrogen scavenging drug, determine whether to administer a nitrogen scavenging drug, treat a nitrogen retention disorder, and predict daily ammonia burden. The use of fasting ammonia levels to predict ammonia exposure provides a significant advantage over previously developed methods by reducing the number of required blood draws and eliminating the confusion associated with conflicting ammonia levels over the course of the day.

[0020] As further disclosed herein, the relationship between ammonia control and neurocognitive outcome was evaluated in UCD patients. Previous research has demonstrated that UCD patients often exhibit lower IQ overall and deficient executive function manifested by difficulty in goal setting, planning, monitoring progress and purposeful problem solving. As set forth herein, it was found that ammonia control with GPB resulted in a significant improvement in executive functions in pediatric patients. Based on these results, methods are provided herein for improving executive function in a pediatric subject with a UCD by administering one or more nitrogen scavenging drugs.

[0021] As further disclosed herein, the relationship between elevated PAA levels and neurological adverse events (AEs) was analyzed. Many of the over 30 reports of administration of NaPBA and/or sodium PAA to humans describe AEs, particularly when administered intravenously. IV administration of PAA to cancer patients was shown previously to result in AEs that included fatigue, dizziness, dysgeusia, headache, somnolence, lightheadedness, pedal edema, nausea, vomiting, and rash (Thibault 1994; Thibault 1995). These AEs correlated with PAA levels from 499 to 1285 μg/mL. Although NaPBA has been used in UCD treatment for over two decades and AEs reportedly associated with PAA are similar to those associated with hyperammonemia, little was known previously about the relationship between PAA levels and neurological AEs in UCD patients. As shown herein, increased PAA levels did not correlate with increased neurological AEs in subjects with UCD. However, PAA levels were associated with an increase in neurological AEs in healthy subjects. Based on these results, methods are provided herein for predicting or diagnosing AEs in a subject by measuring PAA levels. Further provided herein are methods of treating and/or preventing AEs in a subject with elevated PAA levels by administering one or more nitrogen scavenging drugs.

[0022] Provided herein are specific target values for blood ammonia upon which an effective dosage of a nitrogen scavenging drug can be based. In certain embodiments, an effective dosage of a nitrogen scavenging drug may be an initial dosage, subsequent/maintenance dosage, improved dosage, or a dosage determined in combination with other factors. In certain embodiments, the effective dosage may be the same as or different than the initial dosage. In other embodiments, the effective dosage may be higher or lower than the initial dosage. In certain embodiments, methods are provided for adjusting the dose or regimen of a nitrogen scavenging drug to achieve a target ammonia level that is predictive of the average daily ammonia level and/or the highest ammonia value that the subject is likely to experience during the day.

[0023] Using the methods herein, a subject's fasting blood ammonia level may be used as a predictor of daily ammonia burden, average daily ammonia level, and/or highest daily ammonia value. Whether a subject with a nitrogen retention disorder is receiving an optimum dosage of nitrogen scavenging drug may be determined based on predicted daily ammonia exposure. By optimizing the therapeutic efficacy of a nitrogen scavenging drug, the therapeutic dosage of the nitrogen scavenging drug is adjusted so that the subject experiences the desired nitrogen

scavenging effect. In particular, the dose is adjusted so that the subject may experience a normal average daily ammonia level. In certain embodiments, the effective dosage of nitrogen scavenging drug is determined by adjusting (e.g., increasing) a dosage to achieve a fasting blood ammonia level for a subject that is less than or equal to half the ULN for blood ammonia.

[0024] Provided herein in certain embodiments are methods of determining whether the dosage of a nitrogen scavenging drug needs to be increased in a subject with a nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that greater than half the ULN, the dosage of the nitrogen scavenging drug needs to be increased. In certain embodiments, the methods further comprise increasing the dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0025] Provided herein in certain embodiments are methods of determining whether the dosage of a nitrogen scavenging drug needs to be increased in a subject with a nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug needs to be increased. In certain embodiments, the methods further comprise increasing the dosage of the nitrogen scavenging drug if the need exists, and in certain of these embodiments the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0026] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is less than or equal to half the ULN the dosage of the nitrogen scavenging drug is not increased. In certain embodiments, the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0027] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is less than or equal to half the ULN the dosage of the nitrogen scavenging drug is not increased. In certain embodiments, the methods further comprise administering the increased dosage. In certain of these embodiments, administration of the increased dosage results in a normal average daily ammonia level in the subject.

[0028] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased, and if the dosage is significantly less than half the ULN, the dosage of the nitrogen scavenging drug may be decreased. In certain embodiments, the methods further comprise administering the adjusted dosage. In certain of these embodiments, administration of the adjusted dosage results in a normal average daily ammonia level in the subject.

[0029] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder comprising administering an initial dosage of the nitrogen scavenging drug, measuring fasting blood ammonia level, and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, subsequent maintenance dosages of the nitrogen scavenging drug are adjusted to be greater than the initial dosage. In certain embodiments, the methods further comprise administering the increased maintenance dosage, and in certain of these embodiments, administration of the increased maintenance dosage results in a normal average daily ammonia level in the subject.

[0030] Provided herein in certain embodiments are methods of adjusting the dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder to achieve a fasting blood ammonia level that is less than or equal to half the ULN for blood ammonia comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood

ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. After a time period sufficient for the drug to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, greater than 2 weeks), fasting blood ammonia level is measured again and compared to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the dosage of the nitrogen scavenging drug is increased. This process is repeated until a fasting blood ammonia level of less than or equal to half the ULN is obtained.

[0031] Provided herein in certain embodiments are methods for assessing whether a subject with a nitrogen retention disorder is more or less likely to need a dosage adjustment of a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia, wherein a fasting blood ammonia level that is greater than half the value of ULN indicates that the subject is more likely to need a dosage adjustment and a fasting blood ammonia level less than or equal to half the value of ULN indicates that the subject is less likely to need a dosage adjustment.

[0032] Provided herein in certain embodiments are methods of determining whether to administer a nitrogen scavenging drug to a subject with nitrogen retention disorder comprising comparing a fasting blood ammonia level for the subject to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a nitrogen scavenging drug needs to be administered to the subject. In certain embodiments, these methods further comprise administering the nitrogen scavenging drug. In certain embodiments, the subject may not have been administered any nitrogen scavenging drugs prior to the determination. In other embodiments, the subject may have previously been administered a nitrogen scavenging drug other than the one being evaluated. In these embodiments, the methods provided herein can be used to determine whether to administer a new nitrogen scavenging drug to a subject.

[0033] Provided herein in certain embodiments are methods of determining whether to administer a nitrogen scavenging drug to a subject with nitrogen retention disorder comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a nitrogen scavenging drug needs to be administered to the subject. In certain embodiments, these methods further comprise administering the nitrogen scavenging

drug. In certain embodiments, the subject may not have been administered any nitrogen scavenging drugs prior to the determination. In other embodiments, the subject may have previously been administered a nitrogen scavenging drug other than the one being evaluated. In these embodiments, the methods provided herein can be used to determine whether to administer a new nitrogen scavenging drug to a subject.

[0034] Provided herein in certain embodiments are methods for selecting a dosage of a nitrogen scavenging drug for treating a nitrogen retention disorder in a subject based on blood ammonia levels comprising selecting a dosage that results in a fasting blood ammonia level that is less than or equal to half the ULN for blood ammonia. In certain embodiments, selecting the effective dosage is further based on diet, endogenous waste nitrogen excretion capacity, or any combination thereof. In certain embodiments, the methods further comprise administering the selected dosage.

[0035] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder who has previously been administered a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the same dosage or a decreased dosage of the nitrogen scavenging drug. In certain embodiments, administration of an increased dosage results in a normal average daily ammonia level in the subject.

[0036] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder who has previously been administered an initial dosage of a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered a maintenance dosage that is greater than the initial dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the initial dosage or a lower dosage. In certain embodiments, administration of an increased maintenance dosage results in a normal average daily ammonia level in the subject.

[0037] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder comprising administering a nitrogen scavenging drug, then measuring a fasting blood ammonia level for the subject at some point after drug administration and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the subject is administered an increased dosage of the nitrogen scavenging drug. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the subject is administered the original or a lower dosage of the drug.

[0038] Provided herein in certain embodiments are methods of treating a subject with a nitrogen retention disorder comprising administering a first dosage of a nitrogen scavenging drug, measuring a fasting blood ammonia level for the subject, and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a second dosage of a nitrogen scavenging drug that is greater than the first dosage is administered to the subject. A fasting ammonia blood level is measured again in the subject and compared to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a third dosage of a nitrogen scavenging drug that is greater than the second dosage is administered to the subject. This process is repeated until the subject exhibits a fasting blood ammonia level with a value less than or equal to half the ULN.

[0039] Provided herein in certain embodiments are methods of monitoring the efficacy of nitrogen scavenging drug administration in a subject with a nitrogen retention disorder who has previously been administered a nitrogen scavenging drug comprising measuring a fasting blood ammonia level for the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, the previously administered dosage of the nitrogen scavenging drug is considered inadequate to treat the nitrogen retention disorder. If the fasting blood ammonia level has a value that is less than or equal to half the ULN, the previously administered dosage is considered adequate to treat the nitrogen retention disorder. In certain embodiments where the previously administered dosage is considered inadequate to treat the nitrogen retention disorder, the methods provided herein further comprise administering an increased dosage of the nitrogen scavenging drug.

[0040] Provided herein in certain embodiments are methods for monitoring therapy with a nitrogen scavenging drug in a subject having a nitrogen retention disorder comprising measuring

a fasting blood ammonia level from the subject and comparing the fasting blood ammonia level to a ULN for blood ammonia, wherein a fasting blood ammonia level that is greater than half the ULN indicates that the subject is more likely to need a dosage adjustment of the nitrogen scavenging drug, and wherein a fasting blood ammonia level less than or equal to half the ULN indicates that the subject is less likely to need a dosage adjustment.

[0041] A nitrogen retention disorder as used herein refers to any condition associated with elevated blood nitrogen/ammonia levels. In certain embodiments, a nitrogen retention disorder may be a UCD. In other embodiments, a nitrogen retention disorder may be HE.

[0042] A nitrogen scavenging drug as used herein refers to any drug that decreases blood nitrogen and/or ammonia levels. In certain embodiments, a nitrogen scavenging drug may remove nitrogen in the form of PAGN, and in certain of these embodiments the nitrogen scavenging drug may be an orally administrable drug that contains or is metabolized to PAA. For example, a nitrogen scavenging drug may be a PAA prodrug such as PBA or HPN-100, a pharmaceutically acceptable salt of PBA such as NaPBA, or a pharmaceutically acceptable ester, acid, or derivative of a PAA prodrug. In other embodiments, a nitrogen scavenging drug may remove nitrogen via hippuric acid. In certain of these embodiments, a nitrogen scavenging drug may be benzoic acid, a pharmaceutically acceptable salt of benzoic acid such as sodium benzoate, or a pharmaceutically acceptable ester, acid, or derivative of benzoic acid.

[0043] Increasing the dosage of a nitrogen scavenging drug may refer to increasing the amount of drug per administration (e.g., an increase from a 3 mL dosage to a 6 mL dosage), increasing the number of administrations of the drug (e.g., an increase from once-a-day dosing to twice- or three-times-a-day), or any combination thereof.

[0044] A subject that has previously been administered a nitrogen scavenging drug may have been administered the drug for any duration of time sufficient to reach steady state. For example, the subject may have been administered the drug over a period of 2 to 7 days, 1 week to 2 weeks, 2 weeks to 4 weeks, 4 weeks to 8 weeks, 8 weeks to 16 weeks, or longer than 16 weeks.

[0045] In certain embodiments of the methods disclosed herein, the fasting period for obtaining a fasting blood ammonia level is overnight. In certain embodiments, the fasting period is 4 hours or more, 5 hours or more, 6 hours or more, 7 hours or more, 8 hours or more, 9 hours or more, 10 hours or more, 11 hours or more, or 12 hours or more, and in certain embodiments the fasting

period is 4-8 hours, 6-8 hours, or 8-12 hours. During the fasting period, the subject preferably does not ingest any food. In certain embodiments, the subject may also refrain from ingesting certain non-food substances during the fasting period. For example, in certain embodiments the subject does not ingest any supplements and/or nitrogen scavenging drugs during the fasting period. In certain of these embodiments, the subject may nonetheless ingest one or more drugs other than nitrogen scavenging drugs during the fasting period. In certain embodiments, the subject does not ingest any high calorie liquids during the fasting period. In certain of these embodiments, the subject does not ingest any liquids other than water during the fasting period. In other embodiments, the subject may ingest small amounts of low calorie beverages, such as tea, coffee, or diluted juices.

[0046] In certain embodiments of the methods disclosed herein, blood samples used for measuring fasting blood ammonia levels and/or ULN blood ammonias are venous blood samples. In certain embodiments, a blood sample is a plasma blood sample. Any methods known in the art may be used to obtain a plasma blood sample. For example, blood from a subject may be drawn into a tube containing heparin or ethylenediaminetetraacetic acid (EDTA). In certain embodiments, the sample can be placed on ice and centrifuged to obtain plasma within 15 minutes of collection, stored at 2-8°C (36-46°F) and analyzed within 3 hours of collection. In other embodiments, the blood plasma sample is snap frozen, stored at ≤-18°C (≤0°F) and analyzed at a later time. For example, the sample may be analyzed at 0-12 hours, 12-24 hours, 24-48, 48-96 hours after freezing, or within any other timeframe over which the sample has demonstrated stability. In certain embodiments, blood samples are taken in a laboratory or hospital setting. In certain embodiments, a single fasting blood sample is used to measure fasting blood ammonia level. However, in other embodiments, multiple fasting blood samples may be obtained. In certain embodiments, a subject's blood ammonia level may be monitored throughout the day. Further, in certain embodiments, the methods disclosed herein comprise an additional step of obtaining one or more blood samples from a subject prior to or after measuring fasting blood ammonia level.

[0047] In certain embodiments, a blood sample is analyzed immediately after collection. In other embodiments, the blood sample is stored for some period between collection and analysis. In these embodiments, the sample may be stored for less than 1 hour, 1 hour to 6 hours, 1 hour to 12 hours, 1 hour to 24 hours, or 1 hour to 48 hours. In certain of these embodiments, the blood

sample is stored at a temperature between 0-15°C, such as 2-8°C. In other embodiments, the blood sample is stored below 0°C or below -18°C.

[0048] Measurement of ammonia levels in a fasting blood sample is carried out using techniques known in the art. For example, ammonia levels may be measured using a colorimetric reaction or an enzymatic reaction. In certain embodiments, a colorimetric reaction may involve the use of bromophenol blue as an ammonia indicator. In these embodiments, ammonia may react with bromophenol blue to yield a blue dye. In certain embodiments, an enzymatic reaction may involve glutamate dehydrogenase catalyzing the reductive amination of 2-oxoglutarate with NH^{4+} and NADPH to form glutamate and $NADP^{+}$. The formation of $NADP^{+}$ formed is directly proportional to the amount of ammonia present in the blood sample. Therefore, the concentration of ammonia is measured based on a decrease in absorbance.

[0049] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their average daily ammonia level will remain within a normal average daily ammonia level. In certain embodiments, the average likelihood of having a normal daily ammonia value is 80% to 90%. In certain embodiments, one may predict with 95% confidence that a blood ammonia level will fall within a certain range. In certain embodiments, one can predict with 95% confidence that a true probability of predicting normal values based on fasting blood ammonia is between 65% and 93%. In other embodiments, one can predict with 80% confidence that a true probability of predicting normal values based on fasting blood ammonia is at least 70%. In certain embodiments, the average likelihood of predicting normal ammonia value based on fasting blood ammonia is about 84% with 95% confidence that the true probability is between 65% and 93%.

[0050] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their maximum daily blood ammonia level will not exceed 1.5 times the ULN for blood ammonia. In certain of these embodiments, the average likelihood is about 70% to 80%. In certain embodiments, the confidence interval is a 95% confidence interval. In certain embodiments, the average likelihood is about 75% with 95% confidence that the true probability is between 58% and 86%.

[0051] In certain embodiments of the methods disclosed herein, a subject exhibiting a fasting blood ammonia level less than or equal to half the ULN for blood ammonia has an average likelihood within a confidence interval that their maximum daily blood ammonia level will be less than 100 μmol/L. In certain of these embodiments, the average likelihood is 90% to 98%. In certain embodiments, the confidence interval is 95%. In certain embodiments, the average likelihood is about 93% with 95% confidence that the true probability is between 77% and 100%.

[0052] The maximal ammonia value refers to the maximum amount of ammonia that may be detected in a subject following consumption of meals, if repeated measurement of blood ammonia can be instituted to detect such maximum value over an extended period of time. Based on well-controlled clinical trials with repeated blood sampling over 24 hours, the maximum blood ammonia has been observed to occur following the third major meal of the day in the early to mid evening hours (4-8PM, assuming that breakfast is approximately 8AM; see, e.g., Lee 2010; Lichter-Konecki 2011).

[0053] The ULN for blood ammonia typically represents the highest level in the range of normal values, which may be influenced by a variety of factors such as the assay method, types of regents, standard reference samples used, and specifications and calibration of equipment used to perform the measurement. In certain embodiments of the methods disclosed herein, the ULN for blood ammonia is determined for a subject individually. In other embodiments, the ULN for blood ammonia may be based on measurements obtained across a range of subjects (i.e., subjects with UCD or with a particular subtype of UCD, subjects with HE, healthy subjects, etc.). In certain embodiments, the ULN for blood ammonia may represent a standard reference value disclosed in the art, such as a mean ULN developed across a particular subset of subjects. In other embodiments, the ULN for blood ammonia may represent a standard measurement that has been developed by a particular entity that performs blood draws and/or blood evaluations, such as a particular clinical laboratory. In certain embodiments, the ULN is a standard reference value utilized by the same entity that measures the fasting blood ammonia level. In these embodiments, one skilled in the art will appreciate that interpretation of average daily ammonia in subject with a nitrogen retention disorder must be made relative to the reference range of normal values at the laboratory in which the ammonia was measured. Furthermore, the units of ammonia measurement may also vary from lab to lab (e.g., μg/mL or μmoI/L), emphasizing the

importance of interpreting the subject's ammonia levels relative to the ULN at the laboratory in which the measurement was performed. In certain embodiments, the ULN for blood ammonia may be in the range of 26-64 μmol/L. In certain of these embodiments, the ULN for blood ammonia may be in the range of 32-38 μmol/L or 34-36 μmol/L, and in certain of these embodiments the ULN for blood ammonia is 35 μmol/L. In certain embodiments, the ULN for blood ammonia may be in the range of 50-65 μg/mL. In certain of these embodiments, the ULN for blood ammonia may be in the range of 55-63 μg/mL or 57-61 μg/mL, and in certain of these embodiments the ULN for blood ammonia is 59 μg/mL.

[0054] In certain embodiments, the average daily ammonia is the average amount of ammonia an individual may experience during the day, if serial blood sampling were performed for ammonia measurements. In well-controlled clinical studies, it has been established that ammonia fluctuates several fold during the day, depending on the timing of blood draw relative to food and drug intake. Due to these fluctuations, the timing of individual or serial blood sampling should be controlled relative to the timing of food and drug intake. Even serial sampling may not be enough to capture the peaks and troughs of the fluctuating ammonia values, unless samples are taken frequently enough. Therefore, obtaining a simple average of several measurements may provide inadequate or misleading information regarding the total ammonia burden a subject may experience during the day.

[0055] Provided herein are methods to better estimate a subject's average daily ammonia assessed as the area under the curve for 24-hr ammonia (ammonia $AUC_{0\text{-}24hr}$) obtained from adequate and well-spaced samples over 24 hours. This ammonia $AUC_{0\text{-}24hr}$ can be further normalized for the entire actual period of sampling, i.e., ammonia $AUC_{0\text{-}24hr}$ is divided by the sampling period (e.g., 24 hours). For example, if an AUC of 1440 μmol*hr/L is calculated using the trapezoidal rule based on 8-11 ammonia values obtained over 24 hours, then the average daily ammonia value or time-normalized $AUC_{0\text{-}24hr}$ would be equal to 1440 μmol*hr/ml divided by the sampling time of 24 hr, or 60 μmol/L. If the normal reference range at the laboratory which performed the ammonia analysis was 10-35 μmol/L, then the average daily ammonia value for this subject would be approximately 1.71 times the ULN of 35 μmol/L. Similarly, if the ammonia $AUC_{0\text{-}24hr}$ was determined to be equal to 840 μmol*hr/L based on multiple, well-spaced samples over 24 hours and analyzed at the same laboratory, and the sampling period was 24 hours, then the time-normalized $AUC_{0\text{-}24hr}$ would be 35 μmol/L. This corresponds to an

average ammonia or daily ammonia burden within the ULN. Finally, subjects with nitrogen retention disorders such as UCDs may experience a hyperammonemic crisis, which is often defined clinically as a blood level exceeding 100 μmol/L and clinical manifestations of hyperammonemia, which may require intervention to prevent irreversible hard and enable recovery.

[0056] Provided herein are methods of adjusting nitrogen scavenging drug dosage by measuring fasting blood ammonia to minimize the likelihood a subject may experience an ammonia value (Cmax) over 24 hours that exceeds 100 μmol/L. It has been found that 100 μmol/L corresponds to approximately 2-3 times the ULN in most laboratories. Previously, if a subject with a nitrogen retention disorder such as UCD had a blood ammonia level within or slightly above the normal reference range for the laboratory which performed the analysis, the subject was considered to be in good clinical control regardless of the timing of the blood draw in relation to meals and last administration of drug dose. However, it has been shown that a subject with a UCD who has a fasting blood ammonia level between the ULN and 1.5 times the ULN (e.g., 35 to 52 μmol/L) has an average likelihood of only 45% (with a 95% confidence interval of 21% to 70%) that his or her average daily ammonia is within the normal range; an average likelihood of only 35% (with a 95% confidence interval of 13% to 60%) that his or her maximal level of ammonia during the day is less than 1.5 times the ULN (e.g., 52 μmol/L); and an average likelihood of 25% that his or her maximal daily ammonia level exceeds 100 μmol/L during the day. Thus, after measuring a UCD subject's fasting blood ammonia, the dosage of a nitrogen scavenging drug may be progressively increased and/or his or her protein intake progressively decreased until the fasting ammonia value is less than or equal to half of the ULN for the local laboratory in which the ammonia analysis was performed.

[0057] In certain embodiments of the methods disclosed herein, one or more factors other than ammonia level may be taken into consideration when evaluating nitrogen scavenging drug dosage. For example, blood ammonia measurements may be combined with urinary PAGN measurements in determining whether to administer a nitrogen scavenging drug, adjusting the dosage of a nitrogen scavenging drug, or treating a nitrogen retention disorder. US Patent Publication No. 2010/0008859 discloses that urinary PAGN levels correlate more closely to PBA prodrug dosage than plasma PAA, PBA, or PAGN levels, and further discloses that PBA prodrugs are converted to urinary PAGN with a mean efficiency of 60-75%. Therefore, certain

embodiments of the methods disclosed herein comprise an additional step wherein urinary PAGN levels are measured. In certain of these embodiments, calculation of an effective dosage of nitrogen scavenging drug is based in part on a mean 60-75% conversion of PAA prodrug to urinary PAGN. For example, in certain embodiments the methods disclosed herein for determining whether to administer a nitrogen scavenging drug to a subject comprise an additional step of measuring urinary PAGN and calculating an effective initial dosage based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. Similarly, in certain embodiments the methods disclosed herein for adjusting the dosage of a nitrogen scavenging drug comprise an additional step of measuring urinary PAGN and calculating an effective dosage based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%. In certain of these embodiments, the effective dosage is calculated based on a target nitrogen output. In certain embodiments, urinary PAGN may be determined as a ratio of the concentration of urinary PAGN to urinary creatinine. In certain embodiments, urinary PAGN is a factor that is taken into consideration when determining whether to administer or increase the dosage of a nitrogen scavenging drug, i.e., urinary PAGN is evaluated in combination with ammonia level to determine whether to administer or increase the dosage of the drug. In other embodiments, ammonia level alone is used to determine whether to administer or increase the dosage of a nitrogen scavenging drug, and urinary PAGN is simply used to calculate the initial or adjusted dosage.

[0058] One skilled in the art will recognize that a variety of other factors may be taken into consideration when determining the effective dosage of a nitrogen scavenging drug. For example, factors such as diet (e.g., protein intake) and endogenous waste nitrogen capacity (e.g., urea synthesis capacity) may be considered.

[0059] Provided herein in certain embodiments are kits for carrying out the methods disclosed herein. In certain embodiments, kits are provided for determining whether to administer or adjust the dosage of a nitrogen scavenging drug for a subject with a nitrogen retention disorder. The kits disclosed herein may include one or more nitrogen scavenging drugs and/or one or more reagents (e.g., bromophenol blue) or enzymes (e.g., glutamate dehydrogenase) to measure blood ammonia levels in a sample. The kit may additionally include other pigments, binders, surfactants, buffers, stabilizers, and/or chemicals necessary to obtain a blood sample and to

measure the ammonia level in the sample. In certain embodiments, the kits provided herein comprise instructions in a tangible medium.

[0060] One of ordinary skill in the art will recognize that the various embodiments described herein can be combined.

[0061] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

Examples

Example 1: Analysis of predictability of pharmacodynamic ammonia values from fasting ammonia in UCD patients:

[0062] This example demonstrates the relationship between fasting ammonia and the pharmacodynamic (PD) profile of daily ammonia in patients receiving PAA prodrugs for UCDs. Ammonia values vary many-fold over the course of 24 hours in UCD patients. As depicted in Figures 3a and 3b, venous ammonia was measured for 24 hours following one week of dosing with either NaPBA or glycerol phenylbutyrate (GPB). The graphs display ammonia values as mean ±SD over 24 hours, where time zero corresponds to just prior to dosing and breakfast (i.e., fasting state). In view of this variability in daily ammonia levels, a single measurement may not be very informative in determining whether a UCD patient is optimally dosed. The ability to predict the highest potential ammonia a UCD patient may experience during the day and the average 24-hour ammonia from a single measurement such as fasting levels has important practical implications for nitrogen scavenging drug dosing guidelines and patient management.

[0063] Data from two Phase 2 studies and one Phase 3 study comparing ammonia control assessed by 24-hour sampling during steady state treatment with HPN-100 versus NaPBA in 65 UCD patients were used for the analysis. The two Phase 2 studies include protocols UP 1204-003 and HPN-100-005 (Lee 2010; Lichter-Konecki 2011). The Phase 3 study includes protocols from HPN-100-006 (Diaz 2011).

[0064] Ammonia values obtained from different hospital laboratories with different normal ranges were normalized to a standard laboratory range of 9-35 μmol/L. The patient population included a broad range of ages, UCD subtypes, and doses of drug, and is summarized in Table 1 below.

Table 1: UCD demographics in studies UP 1204-003, HPN-100-005, and HPN-100-006:

Gender n (%)	Male	18 (27.7)
	Female	47 (72.3)
Age at screening (years)	N	65
	Mean (SD)	29.46 (15.764)
	Median	24.00
	Range	6.0-75.0
UCD diagnosis n (%)	OTC deficiency	57 (87.7)
	CPS1 deficiency	1 (1.5)
	ASS deficiency	5 (7.7)
	ASL deficiency	1 (1.5)
	Missing	1 (1.5)
Duration of NaPBA treatment (months)	N	63
	Mean (SD)	114.14 (90.147)
	Median	101.00
	Range	0.2-300.0
Daily dose NaPBA	N	64
	Mean (SD)	14.10 (6.255)
	Median	13.50
	Range	1.5-36.0

[0065] Exploratory analysis:

[0066] Several PD parameters for steady-state ammonia were explored: $AUC_{0\text{-}24hr}$, time-normalized AUC, log AUC, maximal ammonia value over 24 hours (Cmax), and average ammonia. Data from 65 subjects from all three studies with steady-state ammonia and fasting ammonia were used. Missing data were imputed per procedures specified in the protocol and statistical analysis plan, except that no imputations were made for subjects who had no PK sampling conducted while on a given study drug.

[0067] Sample collection times of 0-hr (before first daily dose) and 24-hours post-dose (before first daily dose of the following day) were both evaluated as representative of fasting ammonia. No noticeable difference in the shape or quality of the relationship due to the choice of time point was observed.

[0068] The relationship between fasting ammonia and pharmacokinetic profile was evaluated separately for HPN-100 and NaPBA, with no apparent difference in the strength or magnitude of

the relationship. Therefore, all data from both HPN-100 and NaPBA treatments were used and conclusions regarding fasting ammonia pertain to both HPN-100 and NaPBA.

[0069] The relationships between (1) fasting ammonia and $AUC_{0\text{-}24hr}$ and (2) fasting ammonia and maximum observed ammonia (Cmax) were visually explored for the whole population. The effects of the following covariates were also observed: age, weight, gender, and dietary protein intake. A positive and strong relationship was observed between fasting ammonia and $AUC_{0\text{-}24hr}$, with increasing fasting ammonia being associated with higher $AUC_{0\text{-}24hr}$ and maximum observed ammonia (Figure 2).

[0070] Prediction of $AUC_{0\text{-}24hr}$ through GEE Modeling:

[0071] The aim of this modeling was to predict average daily or highest achieved ammonia based on the subject's fasting ammonia. In order to take into account the differences in normal ranges at different laboratories, all ammonia values were normalized to a reference range of 9-35 μmol/L, and the predictions were referenced to the ULN rather than a fixed value.

[0072] Generalized Estimating Equations (GEE) were used to model the predictive ability of fasting ammonia against various ammonia PD properties. GEE methodology can be used to analyze repeated measures of categorical data, in which the repeated measures are assumed to be correlated (Liang 1986). The model allows for the specification of the assumed correlation structure without the knowledge of the magnitude of the correlation.

[0073] The 24-hour ammonia profile was divided into ordered categories using a variety of endpoints and cutpoints as follows:

1) AUC [0-1.0*ULN, >1.0*ULN];
2) AUC [0-1.5*ULN, >1.5*ULN];
3) Cmax [0-1.0*ULN, >1.0*ULN];
4) Cmax [0-1.5*ULN, >1.5*ULN]; and
5) Cmax [0-100] μmol/L.

[0074] Three levels of fasting ammonia were considered in separate models as input:

1) [0-0.5*ULN];
2) [>0.5*ULN-<1.0 ULN]; and
3) [>1.0*ULN-1.5*ULN].

[0075] Using Statistical Analysis Software (SAS) Proc Genmod, generalized linear models were fit with a logit link function. Pre-dose fasting ammonia was the only predictor variable in

the model. The repeated nature of the data (two study periods per subject) was modeled using GEE with exchangeable correlation matrix. ULN for fasting ammonia was set at 35 μmol/L. ULN for AUC over 24 hours was taken as 840 (35 μmol/L * 24 hours); i.e., the AUC which corresponds to an average daily ammonia less than or equal to 35 μmol/L, which was the normalized ULN among the participating study sites and is derived by dividing the 24-hour area under the curve by the sampling time of 24 hours. The GEE model was bootstrap-resampled 1,000 times according to the method outlined in Davison, A.C. & Hinkley, D.V., Bootstrap Methods and their Application, Cambridge University Press, London (1997), pp.358-362. The results of these models are shown in Table 2 below.

Table 2: Summary of results from GEE model to predict ability of fasting ammonia against various ammonia PD properties:

Model #	Fasting ammonia level	Ammonia PK outcome	Probability of outcome in category	Bootstrap 95% c.i.	Bootstrap 80% c.i.	Bootstrap pred. error rate* (%)
1	[0-0.5 ULN]	AUC in 24 hours [0-1.0 ULN]	0.84	0.67, 0.93	0.71, 0.89	11.5
2		AUC in 24 hours [0-1.5 ULN]	Did not converge			
3		Cmax observed [0-1.0 ULN]	0.53	0.38, 0.65	0.42, 0.61	45.8
4		Cmax observed [0-1.5 ULN]	0.76	0.61, 0.86	0.66, 0.82	23.3
5		Cmax observed [0-100]	0.93	0.78, 1.00	0.85, 0.97	5.7
6	[0-<1.0 ULN]	AUC in 24 hours [0-1.0 ULN]	0.58	0.42, 0.73	0.48, 0.68	42.8
7		AUC in 24 hours [0-1.5 ULN]	0.88	0.78, 0.97	0.82, 0.94	11.1
8		AUC in 24 hours [0-2 ULN]	0.97	0.90, 1.00	0.93, 1.00	2.2
9		Cmax observed [0-	0.21	0.11, 0.38	0.14, 0.33	20.0

		1.0 ULN]				
10		Cmax observed [0-1.5 ULN]	0.52	0.35, 0.66	0.42, 0.61	46.0
11		Cmax observed [0-2.0 ULN]	0.74	0.62, 0.85	0.91, 1.00	27.2
12		Cmax observed [0-100]	0.95	0.88, 1.00	0.66, 0.81	4.3
13	[>1.0-1.5 ULN]	AUC in 24 hours [0-1.0 ULN]	0.45	0.24, 0.71	0.30, 0.63	43
14		AUC in 24 hours [0-1.5 ULN]	Did not converge			
15		AUC in 24 hours [0-2 ULN]	0.80	0.49, 0.99	0.63, 0.92	27
16		Cmax observed [0-1.0 ULN]	Did not converge			
17		Cmax observed [0-1.5 ULN]	0.35	0.16, 0.58	0.23, 0.51	33
18		Cmax observed [0-2.0 ULN]	Did not converge			
19		Cmax observed [0-100]	Did not converge			

[0076] From Table 2 above, we can conclude that in the population of UCD patients described in Table 1, we can be 95% confident that, given a fasting ammonia less than or equal to half the ULN, the true probability of having an AUC in the range [0-840] is on average 84%, at least 67%, and as high as 93%.

[0077] Row 1 of Table 2 above suggests that a UCD patient with a fasting ammonia of 17 μmol/L as determined by a laboratory with a normal reference range of 9-35 μmol/L (i.e., a fasting ammonia in the range [0-0.5 ULN]) has an 84% chance (with a 95% confidence interval of 67% to 93%) of having a time normalized $AUC_{0\text{-}24hr}$ in the normal range [$AUC_{0\text{-}24hr}$ of 0-840 or an average daily ammonia of 35 μmol/L], a 76% chance (with a 95% confidence interval of 61% to 86%) of having a Cmax of less than l.5 ULN, and a 93% chance (with a 95% confidence

interval of 78% to 100%) of never having an ammonia of more than 100 μmol/L. Therefore, this patient would be optimally controlled and unlikely to suffer from high ammonia during the day.

[0078] This Example shows that fasting ammonia correlates strongly with daily ammonia exposure, assessed as a daily average or as maximal daily concentration, and that a target fasting value which does not exceed half of the upper level of normal for the local lab appears to be a clinically useful as well as practical predictor of ammonia values over 24 hours as well. Furthermore, this Example shows that a subject with a fasting ammonia in the range 0-0.5 ULN has an 84% chance of having an $AUC_{0\text{-}24hr}$ in the normal range (0-840 or an average daily ammonia of 35 μmol/L).

Example 2: Selecting and adjusting HPN-100 dosage based on fasting blood ammonia levels in a patient with UCD:

[0079] Patient A is an adult with UCD being managed with amino acid supplements and dietary protein restriction only. Patient A consumes neither his supplements nor food for approximately 8 hours prior to a fasting morning blood draw. A venous blood draw is performed, and fasting blood ammonia level is determined to be 52 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia in the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient A's fasting blood ammonia level of approximately 1.5 times the ULN represents only a 45% chance on average of having an average ammonia during the day within the normal range. Thus, the ratio of fasting blood ammonia level to ULN for blood ammonia indicates that Patient A will benefit from treatment with a nitrogen scavenging drug.

[0080] The physician elects to treat Patient A with HPN-100. Initial dosage is determined based on body surface area or as otherwise instructed according to HPN-100 drug labeling. Patient A's body surface area is 1.4 m^2, and therefore the initial dosage is determined to be 9 mL per day or 3 mL TID, which is approximately 60% of the maximum allowed dosage per HPN-100 label. Patient A is treated with 9mL/day of HPN-l00 for at least 7 days, and returns for an additional blood draw. The fasting blood ammonia level at this time is 33 μmol/L, which is slightly below the ULN and falls into the range of 0.5 to 1.0 times normal. Patient A's blood ammonia level is monitored throughout the day after administration of a 3 mL dose of HPN-l00 with each meal. It is observed that Patient A's maximum ammonia reaches 95 μmol/L after

dinner with an average daily ammonia of 66 μmol/L, which is almost two times the upper normal range. Therefore, Patient A's dosage of HPN-100 is increased by approximately one-third to 12 mL total or 4 mL TID. Patient A returns after at least 7 days of treatment with HPN-100. Patient A's fasting ammonia level is 15 μmol/L, which is less than half of the ULN range. It is determined that Patient A has reached satisfactory ammonia control.

[0081] It is expected that if Patient A adheres to his prescribed diet, his maximal daily ammonia is not expected to exceed approximately 52 μmol/L, i.e., approximately 1.5 times the ULN, with an average likelihood of 75% with 95% confidence. The average ammonia level during the day is expected to remain within normal range with greater than 84% likelihood and 95% confidence. Moreover, Patient A's maximal daily ammonia is highly unlikely to reach 100 μmol/L during the day.

Example 3: Adjusting HPN-100 dosage based on fasting blood ammonia levels in a patient with UCD:

[0082] Patient B is an 11-year UCD patient receiving 24 pills of BUPHENYL® per day, amino acid supplements, and restricted dietary protein intake. Patient B does not consume BUPHENYL®, supplements, or food for approximately 6 hours prior to a fasting morning blood draw. A venous blood draw is performed, and fasting blood ammonia level is determined to be 40 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia for the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient B's fasting blood ammonia level falling between 1 and 1.5 times the ULN represents a 55% chance of having an average ammonia during the day that is greater than the normal range, and as high as a 65% chance that her ammonia will go above 52 μmol/L or 1.5 times ULN during the day.

[0083] Based on discussion with the patient and her mother, the physician suspects that Patient B is noncompliant with her medication, and decides to change her to HPN-100. The initial dosage is determined based on the amount of BUPHENYL® Patient B was receiving, and it is determined that Patient B needs to take 10.5 mL of HPN-l00 per day. Patient B is treated with 3.5mL of HPN-100 3 times a day for at least 7 days, and returns for additional blood draws. Her fasting blood ammonia level at this time is 17 μmol/L, which is below the ULN and falls into the range of 0 to 0.5 times normal. It is determined that Patient B has reached satisfactory ammonia control.

[0084] It is expected that if Patient B adheres to her prescribed diet, her maximal daily ammonia will not go above approximately 50 μmol/L, which is less than 1.5 times the ULN. Her average ammonia level during the day is expected with greater than 84% average likelihood to remain within normal range. Moreover, there is only a small chance (7%) that Patient B's maximal daily ammonia will exceed 100 μmol/L during the day.

Example 4: Selecting and adjusting sodium benzoate dosage based on fasting blood ammonia levels in a patient with UCD:

[0085] Patient C is an adult UCD patient who is allergic to PBA and is therefore being managed with amino acid supplements and dietary protein restriction only. Patient C complains of chronic headache and frequent nausea. Patient C consumes neither his supplements nor food for approximately 8 hours prior to a fasting morning blood draw. A venous blood draw is performed, and fasting blood ammonia level is determined to be 77 μmol/L. This fasting blood ammonia level is compared to the ULN for blood ammonia for the laboratory performing the blood draw, which is 35 μmol/L. Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient C's fasting blood ammonia level of approximately 2 times the ULN represents a high likelihood of ammonia levels going over 100 μmol/L during the day. Thus, the ratio of fasting blood ammonia level to ULN for blood ammonia indicates that Patient C will benefit from treatment with a nitrogen scavenging drug.

[0086] The physician decides to treat Patient C with 15 g of sodium benzoate per day since the patient is allergic to PBA. Patient C is treated with 15 g/day of sodium benzoate for at least 7 days, and returns for additional blood draws. Fasting blood ammonia level at this time is 35 μmol/L, which is equal to the ULN. Patient C's dosage of sodium benzoate is increased by approximately 30% to 18 grams per day. After at least 7 days of treatment, Patient C's fasting ammonia level is 15 μmol/L, which is less than half of the ULN. It is determined that Patient C has reached satisfactory ammonia control.

[0087] It is expected that if Patient C adheres to his prescribed diet and medication, his maximal daily ammonia will not exceed approximately 52 μmol/L, which is approximately 1.5 times the ULN. His average ammonia level during the day is expected with greater than 80% likelihood to remain within normal range. Moreover, Patient C's maximal daily ammonia is highly unlikely to reach 100 μmol/L during the day.

Example 5: Evaluation of the effect of ammonia control on neurocognitive outcome:

[0088] It has been shown that UCD patients are likely to suffer from diminished intelligence and impaired neurocognitive functions (Kirvitsky 2009). These neuropsychological impairments have been attributed to repeated episodes of acute hyperammonemia interspersed on chronically elevated ammonia. Abnormalities in neuropsychological function and/or brain imaging have been detected even in UCD patients with mild disorders who exhibit normal IQ and/or appear clinical normal (Gropman 2008a; Gropman 2008b). Therefore, it was hypothesized that maintaining average daily ammonia within normal limits and thereby reducing the long term ammonia burden could result in improved cognition.

[0089] The relationship between reducing ammonia burden by maintaining fasting ammonia at or close to half ULN and neuropsychological outcomes in pediatric UCD patients was explored in clinical trials. Eleven pediatric patients ages 6-17 were enrolled in short term switch over comparison of NaPBA and HPN-100 in controlling ammonia. These patients underwent 24-hr serial sample collection in a confined setting where the last sample at 24 hr was considered fasting and under supervision of the study personnel. At the end of treatment with HPN-100 the average fasting ammonia at 24-hr time point was 15.5 μmol/L or less than half ULN, indicating good clinical control. These 11 patients along with another 15 pediatric patients were enrolled in two long term studies and received HPN-100 for 12 months, during which monthly fasting ammonia were collected. At the time of enrollment and at the end of the study, all patients underwent assessment for neuropsychological outcomes including the following: BRIEF (Behavior Rating Inventory of Executive Function) to assess day-to-day executive functioning, CBCL (Child Behavior Checklist) to evaluate internalizing (e.g., mood/anxiety) and externalizing behaviors, and WASI (Wechsler Abbreviated Scale of Intelligence) to estimate of intellectual ability.

[0090] During the 12 month treatment with HPN-100, pediatric UCD patients experienced fewer episodes of acute hyperammonemia than in the 12 months preceding enrollment (5 episodes during the study versus 9 before enrollment), with peak ammonia dropping from a mean of 233 μmol/L before enrollment to 166 μmol/L during the study. Fasting ammonia remained controlled and monthly averages were at or close to half ULN, ranging from 17 to 22 μmol/L. Although patients had been instructed to remain fasting before monthly study visits, some ammonia samples were taken in a non-fasted state, resulting in average monthly ammonia of slightly above half ULN.

[0091] In pediatric patients, WASI and CBCL scores were stable in comparison to baseline. The majority of the BRIEF subscales at baseline were at or close to 65, consistent with borderline and/or clinically significant dysfunction. Among 22 pediatric subjects who completed the neuropsychological testing at 12 months, all BRIEF domains were improved (lower T scores) with means (SD) at end of study compared to baseline for Behavioral Regulation Index 53.7 (9.79) vs. 60.4 (14.03) ($p<0.05$); Metacognition Index 57.5 (9.84) vs. 67.5 (13.72) ($p<0.001$), and Global Executive Scale 56.5 (9.71) vs. 66.2 (14.02) ($p<0.001$).

[0092] The significant improvement in executive functions in this group of pediatric UCD patients indicates the importance of long term ammonia control and achieving target levels of fasting ammonia.

Example 6: Correlation of elevated PAA levels to neurological AEs in UCD and healthy subjects:

[0093] Elevated plasma levels of PAA may cause symptoms that mimic those associated with hyperammonemia, including headache, nausea, somnolence, etc. Since such symptoms are common and nonspecific, an ammonia level below half the upper limit of normal in a subject with a nitrogen retention disorder who exhibits such symptoms and is receiving a PAA prodrug would prompt a physician to check plasma PAA levels.

[0094] The relationship between elevated PAA levels and neurological AEs was evaluated in three populations: (1) 130 healthy adults dosed with 4 to 12 mL TID of GPB in a thorough QTc study, (2) 54 adult and 11 pediatric UCD patients (ages 6-17) enrolled in one of 3 protocols involving short term (2-4 week) switchover comparisons of NaPBA vs. GPB, and (3) 77 patients enrolled in two nearly identical 12-month GPB treatment protocols. In populations 1 and 2, maximal PAA (i.e., Cmax) levels were analyzed in relation to neurological AEs as defined by MEDDRA using an Exact non-parametric Mann-Whitney test and Generalized Estimating Equations (GEE) with a logit link function and effects for dose and PAA level. The relationship between PAA levels and the occurrence of the AEs reported by Thiebault was also explored in population 3.

[0095] No statistically significant relationship was observed between neurological AEs and PAA levels for either GPB or NaPBA. The odds ratio of a neurological AE occurring for each 20 μg/mL increase in PAA levels for the two drugs combined was 0.95, very close to 1. Thus, among UCD patients dosed with HPN-100 or NaPBA over the ranges used in these studies,

increasing levels of PAA (ranging up to 244 μg/mL) were not associated with an increase in neurological AEs. Similarly, in population 3, PAA levels did not increase over time and exhibited no apparent relationship to neurological AEs, which also did not increase in frequency over time. The pediatric patient with the highest PAA level (410 μg/mL) did not report neurological AEs close to the timing of the blood draw.

[0096] Unlike UCD subjects, healthy adult volunteers who reported a nervous system AE had statistically significantly higher PAA C_{max} levels than those who did not. While this analysis in healthy adults is compromised by the fact that PAA levels were not always available at the time of occurrence of the AEs, as well as by the small sample size in the higher dose groups, the odds ratio of 1.75 (p=0.006) suggests that increasing levels of PAA are associated with increased probability of experiencing a nervous system AE among healthy adults. AEs reported by healthy adults generally began within 36 hours of dosing and, among those adults who remained on study, most resolved with continued dosing.

[0097] A significant relationship between PAA levels and occurrence of neurological AEs, which generally resolved with continued dosing, was detected in healthy volunteers. Unlike in healthy adults, PAA C_{max} did not correlate with nervous system AEs in UCD patients over a similar range of doses and PAA levels. These findings may reflect metabolic differences among the populations (e.g., UCD patients exhibit high glutamine levels compared with healthy humans) and/or metabolic adaptation with continued dosing.

[0098] Population PK model building was performed on 65 UCD patients who participated in the short-term switchover Hyperion studies using NONMEM (version 7.2) based on 2981 ([PBA], [PAA], [PAGN], and urine PAGN [UPAGN])) data points from 53 adult and 11 pediatric UCD patients (ages 6-17) who participated in 3 switchover studies of NaPBA and GPB. The median GPB dose, expressed as grams of PBA per m2, was 8.85 and 7.01 for pediatric and adult subjects, respectively. Diagnostic plots and statistical comparisons were used to select among candidate models, and covariates were assessed by graphical analyses and covariate modeling. Using the final popPK model and parameter estimates, Monte Carlo simulations were performed in ~1000 virtual patients for a range of NaPBA and GPB doses to predict systemic metabolite exposure and UPAGN output.

[0099] The final model that best fit the data was characterized by (a) partial conversion of PBA to PAGN prior to reaching the systemic circulation, (b) saturable conversion of PAA to PAGN

(Km ~161ug/ml), and (c) ~60% slower PBA absorption when delivered as GPB vs. NaPBA. Body surface area (BSA) was a significant covariate such that metabolite clearance was proportionally related to BSA. Fractional presystemic metabolism of PBA was higher for adults than for pediatric patients receiving GPB (43% vs. 14%), whereas the reverse was true for NaPBA (23% vs. 43%). Predicted median PAA exposure based on simulated GPB dosing at the PBA equivalent of 13g/m2 of NaPBA was ~13%-22% lower in adults than NaPBA (Cmax = 82 vs. 106 μg/mL; AUC_{0-24} = 649 vs. 829 μg.h/m) and ~13% higher in pediatric subjects ages 6-17 than NaPBA (Cmax = 154 vs. 138 μg/mL; AUC_{0-24} = 1286 vs. 1154 μg.h/ml); predicted upper 95th percentile PAA exposure was below 500 μg/mL and 25%-40% lower for adult subjects on GPB versus NaPBA and similar for pediatric subjects. Simulated dosing at the PBA equivalent of ~5g/m^2 of NaPBA yielded similar and less variable PAA exposure for both drugs and for pediatric and adult patients. Recovery of PBA as UPAGN was very similar whether delivered orally as GPB or NaPBA.

[00100] These findings based on PopPK modeling and dosing simulations suggest that while most patients treated with PAA prodrugs including NaPBA or HPN-100 will have PAA levels below those reportedly associated with toxicity and while no relationship between PAA levels and neurological AEs was found on a population basis, individual patients exhibiting symptoms such as headache or nausea might be suffering from either hyperammonemia or high PAA levels and that a fasting ammonia level equal to or below half the upper limit of normal would prompt the physician to check plasma PAA levels.

[00101] As stated above, the foregoing is merely intended to illustrate various embodiments of the present invention. The specific modifications discussed above are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein are incorporated by reference as if fully set forth herein.

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What is claimed is:

1. A method for adjusting the dosage of glyceryl tri-[4-phenylbutyrate] in a subject being treated for a urea cycle disorder who has previously been administered an initial dosage of glyceryl tri-[4-phenylbutyrate], the method comprising:

(a) measuring a fasting plasma ammonia level for the subject;

(b) comparing the fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate], wherein the adjusted dosage is greater than the initial dosage if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

2. A method of treating a subject with a urea cycle disorder who has previously been administered an initial dosage of glyceryl tri-[4-phenylbutyrate], the method comprising:

(a) measuring a fasting plasma ammonia level for the subject;

(b) comparing the fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate] that is greater than the initial dosage if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

3. A method of administering glyceryl tri-[4-phenylbutyrate] to a subject having a urea cycle disorder, the method comprising:

(a) measuring a first fasting plasma ammonia level for the subject;

(b) comparing the first fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an initial dosage of glyceryl tri-[4-phenylbutyrate] to the subject if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

4. The method of claim 1 or 2, wherein administering the adjusted dosage of glyceryl tri-[4-phenylbutyrate] produces a normal average daily ammonia level in the subject.

5. The method of claim 1 or 2, further comprising repeating steps (a) to (c) until the subject exhibits a fasting plasma ammonia level at or below half the upper limit of normal for plasma ammonia level.

6. The method of claim 3, further comprising:

(d) measuring a second fasting plasma ammonia level for the subject;

(e) comparing the second fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(f) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate] that is greater than the initial dosage if the second fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

7. The method of any of claims 1-3, wherein the upper limit of normal for plasma ammonia level is 35 μmol/L.

8. The method of any of claims 1-3, wherein the upper limit of normal is specific to the laboratory in which the fasting plasma ammonia level is measured.

9. The method of any of claims 1-3, further comprising the step of determining an upper limit of normal for plasma ammonia level for the subject prior to step (b).

10. The method of claim 1 or 2, wherein the adjusted dosage is calculated by:

(i) measuring urinary phenylacetyl glutamine (PAGN) output; and

(ii) calculating an effective adjusted dosage of glyceryl tri-[4-phenylbutyrate] based on the urinary PAGN output, wherein the effective adjusted dosage is calculated based on a mean conversion of glyceryl tri-[4-phenylbutyrate] to urinary PAGN of 60 to 75%.

11. The method of claim 3, wherein the initial dosage is calculated by:

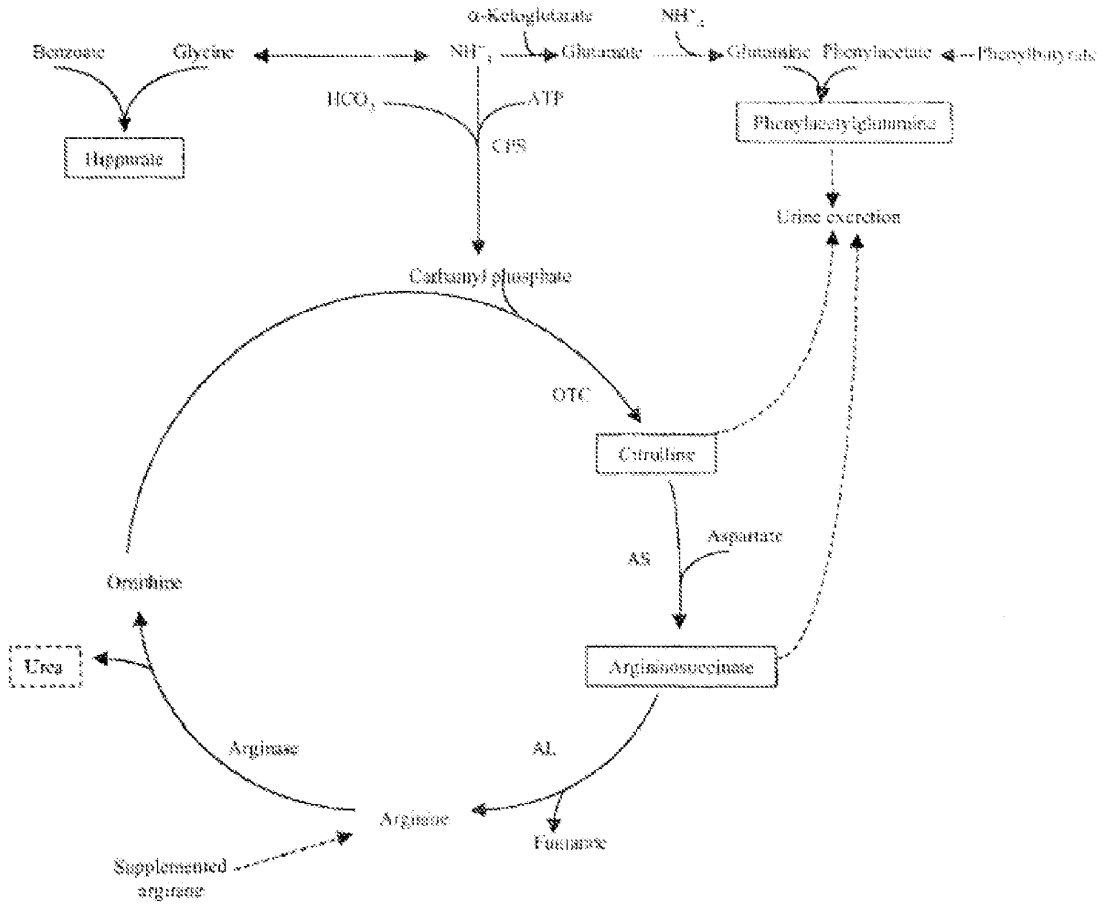
(i) determining a target urinary phenylacetyl glutamine (PAGN) output; and

(ii) calculating an effective initial dosage of glyceryl tri-[4-phenylbutyrate] based on a mean conversion of glyceryl tri-[4-phenylbutyrate] to urinary PAGN of 60 to 75%.

ABSTRACT

The present disclosure provides methods for evaluating daily ammonia exposure based on a single fasting ammonia blood level measurement, as well as methods that utilize this technique to adjust the dosage of a nitrogen scavenging drug, determine whether to administer a nitrogen scavenging drug, and treat nitrogen retention disorders.

Figure 1


α-Ketoglutarate
Benzoate
Glycine
Glutamate
Glutamine
Phenylacetate
Phenylbutyrate
ATP
CPS
Hippurate
Phenylacetylglutamine
Urine excretion
Carbamyl phosphate
OTC
Citrulline
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Ornithine
Urea
Argininosuccinate
Arginase
AL
Arginine
Fumarate
Supplemented arginine

Figure 2

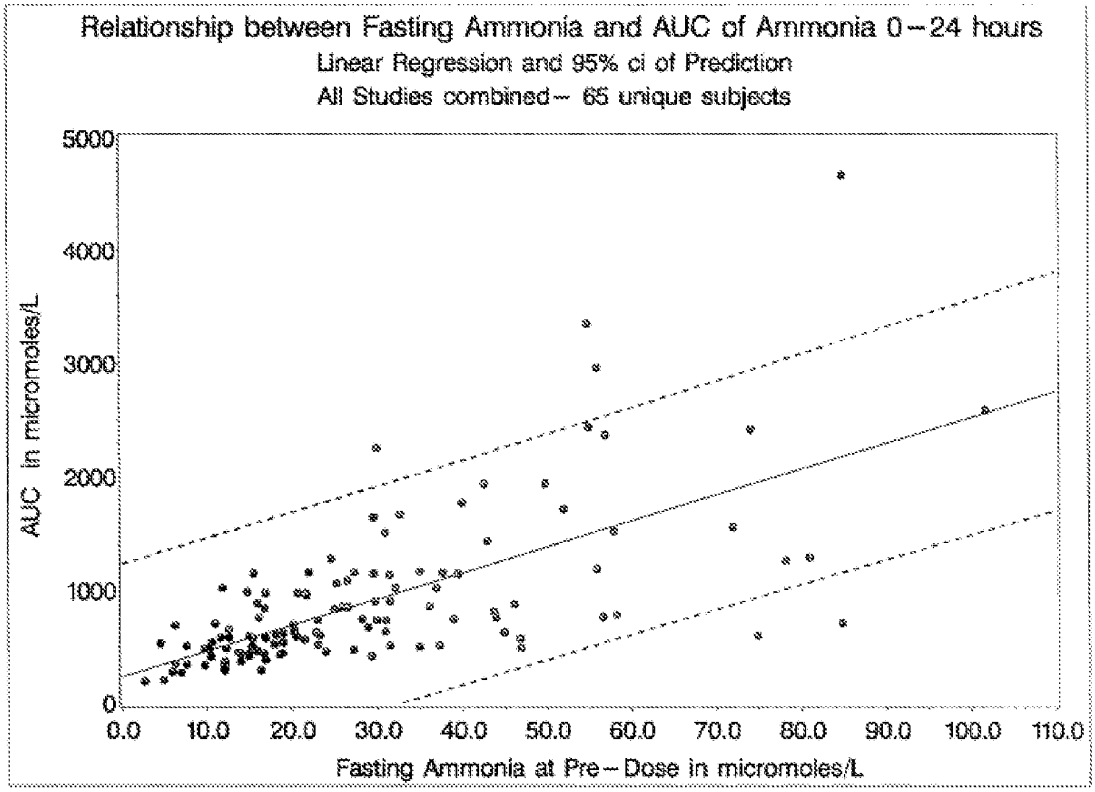
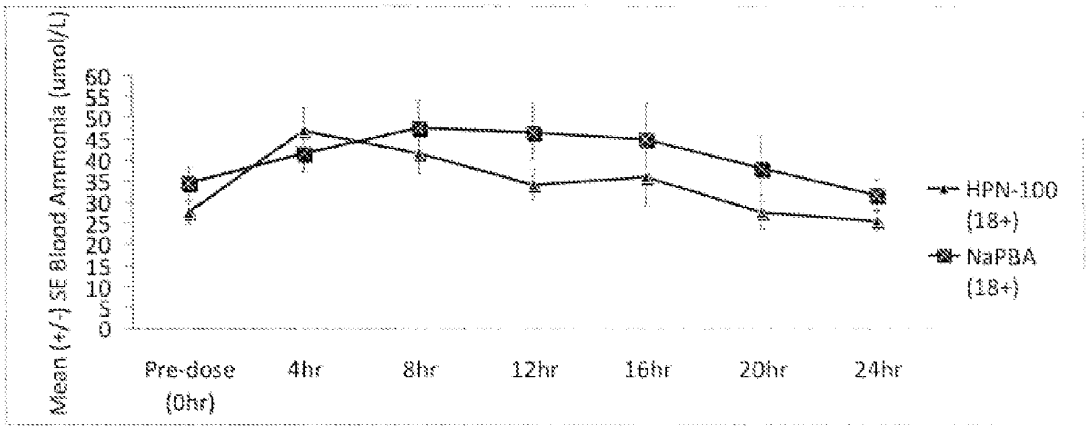
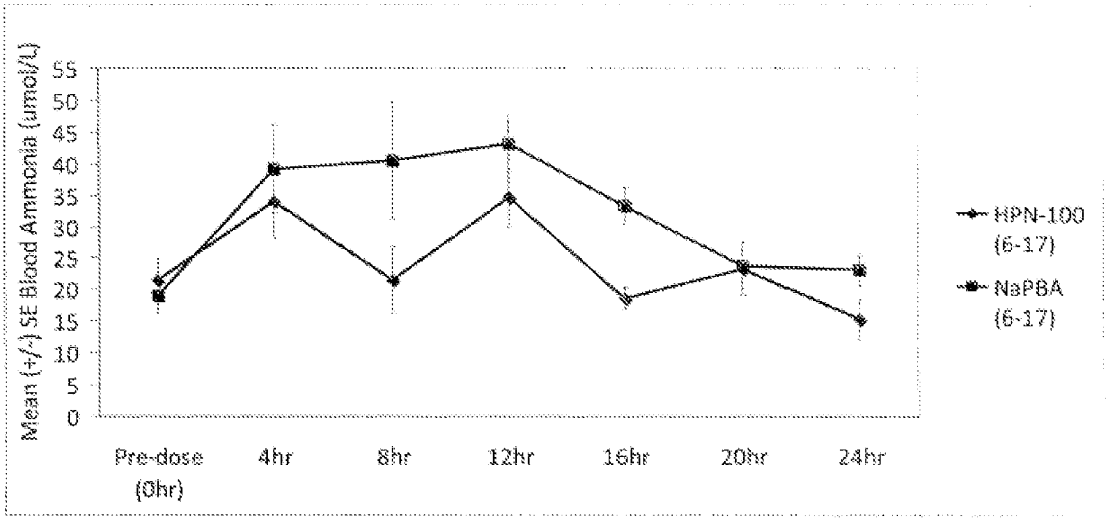

Relationship between Fasting Ammonia and AUC of Ammonia 0 – 24 hours
Linear Regression and 95% ci of Prediction
All Studies combined – 65 unique subjects
AUC in micromoles/L
5000
4000
3000
2000
1000
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
Fasting Ammonia at Pre – Dose in micromoles/L

Figure 3

A.


Mean (+/-) SE Blood Ammonia (umol/L)
60
55
50
45
40
35
30
25
20
15
10
5
0
Pre-dose (0hr)
4hr
8hr
12hr
16hr
20hr
24hr
HPN-100 (18+)
NaPBA (18+)

B.


Mean (+/-) SE Blood Ammonia (umol/L)
55
50
45
40
35
30
25
20
15
10
5
0
Pre-dose (0hr)
4hr
8hr
12hr
16hr
20hr
24hr
HPN-100 (6-17)
NaPBA (6-17)

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Michael J. Wise/Amy Candeloro
Attorney Docket Number:	79532.8003.US03

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	98	98
Utility Search Fee	2111	1	310	310
Utility Examination Fee	2311	1	125	125
Pages:				
Claims:				
Multiple dependent claims	2203	1	230	230
Miscellaneous-Filing:				
Late filing fee for oath or declaration	2051	1	65	65

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**828**

Electronic Acknowledgement Receipt	
EFS ID:	15032365
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Michael J. Wise/Amy Candeloro
Filer Authorized By:	Michael J. Wise
Attorney Docket Number:	79532.8003.US03
Receipt Date:	22-FEB-2013
Filing Date:	
Time Stamp:	19:41:23
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$828
RAM confirmation Number	7851
Deposit Account	502586
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)

1	Application Data Sheet	2013-02-22_ADS_795328003US3.pdf	1433271 d695c739cd56e15d0ca497749500582c69d88823	no	6
Warnings:					
Information:					
2		2013-02-22_Specification_Drawings_795328003US3.pdf	282787 672cd44950511fec0bd0d195395d6a8b0a97caa2	yes	38

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	32
	Claims	33	34
	Abstract	35	35
	Drawings-other than black and white line drawings	36	38

Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	38393 533bd9fb819ca7c115e941d975981bb5dadc7f78	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1754451		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 13775000 Document Date: 02/22/2013

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/775,000	02/22/2013	1736	828	79532.8003.US03	11	3

CONFIRMATION NO. 7929

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

FILING RECEIPT

OC000000059920059

Date Mailed: 03/20/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

Applicant(s)

HYPERION THERAPEUTICS, INC., South San Francisco, CA

Assignment For Published Patent Application

HYPERION THERAPEUTICS, INC., South San Francisco, CA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a DIV of 13/417,137 03/09/2012 PAT 8404215 *
which claims benefit of 61/542,100 09/30/2011
and claims benefit of 61/564,668 11/29/2011
(*)Data provided by applicant is not consistent with PTO records.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access - A proper **Authorization to Permit Access to Application by Participating Offices** (PTO/SB/39 or its equivalent) has been received by the USPTO.

If Required, Foreign Filing License Granted: 03/15/2013

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/775,000**

Projected Publication Date: To Be Determined - pending completion of Corrected Papers

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Preliminary Class

423

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)	Application Number 13775000	Filing Date
	Applicant(s) Bruce Scharschmidt	

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
1	1					
2	1					
3	1					
4		2				
5		2				
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9		3				
10		2				
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50						
Total Indep	3		0		0	
Total Depend	17	↵	0	↵	0	↵
Total Claims	20		0		0	

CLAIMS	*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend
51						
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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/775,000	02/22/2013	Bruce Scharschmidt	79532.8003.US03

CONFIRMATION NO. 7929

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

FORMALITIES LETTER

OC000000059920060

Date Mailed: 03/20/2013

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings have a line quality that is too light to be reproduced (weight of all lines and letters must be heavy enough to permit adequate reproduction) or text that is illegible (reference characters, sheet numbers, and view numbers must be plain and legible) see 37 CFR 1.84(l) and (p)(1)); See Figure(s) 2, 3.
  - The drawings submitted to the Office are not electronically reproducible because portions of figures 1 are missing and/or blurry.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Items Required To Avoid Processing Delays:

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  All
  Applicant may submit the inventor's oath or declaration at any time before the Notice of Allowance and Fee(s) Due, PTOL-85, is mailed.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/atesfaye/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/775,000

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	x 40 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				390			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1120		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: BRUCE SCHARSCHMIDT ET AL.	CONFIRMATION NO.: 7929
APPLICATION NO.: 13/775,000	ART UNIT: 1736
FILING DATE: FEBRUARY 22, 2013	
FOR: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Notice to File Corrected Application Papers mailed on March 20, 2013, applicants submit the following:

☒ an executed Declaration of Inventorship;

☒ Replacement Drawings (3 sheets); and

☒ an Information Disclosure Statement (Form PTO/SB/08a) with cited references.

No fees are believed to be due with this response. However, the Commissioner is authorized to charge Deposit Account No. 50-2586 for any fee believed to be due.

Dated: April 18, 2013

Respectfully submitted,

PERKINS COIE LLP

By: /Patrick D. Morris/
Patrick D. Morris, Ph.D.
Reg. No. 53,351

Correspondence Address:
Customer No. 34055
Perkins Coie LLP
Patent - LA
P.O. Box 1208
Seattle, WA 98111-1208
Phone: (310) 788-9900
Fax: (206) 332-7198

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	**METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS**

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 13/775,000 filed on February 22, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I am aware of and acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

LEGAL NAME OF INVENTOR: **Masoud Mokhtarani**

Signature: M Mok **Date:** 3/15/2013

Perkins Coie

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	**METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS**

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 13/775,000 filed on February 22, 2013.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I am aware of and acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

LEGAL NAME OF INVENTOR: **Bruce Scharschmidt**

Signature: Bruce F Scharschmidt **Date:** 5/15/13

Perkins Coie

Figure 1

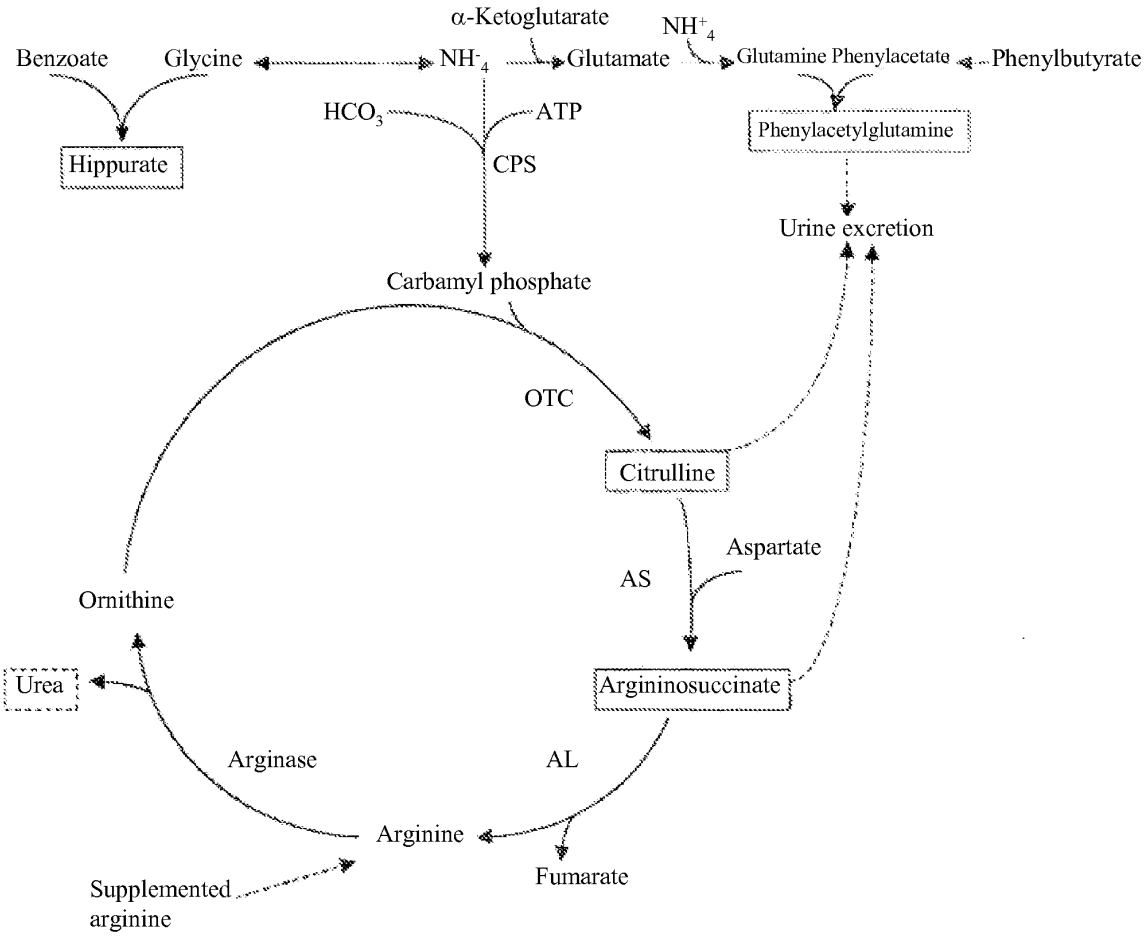
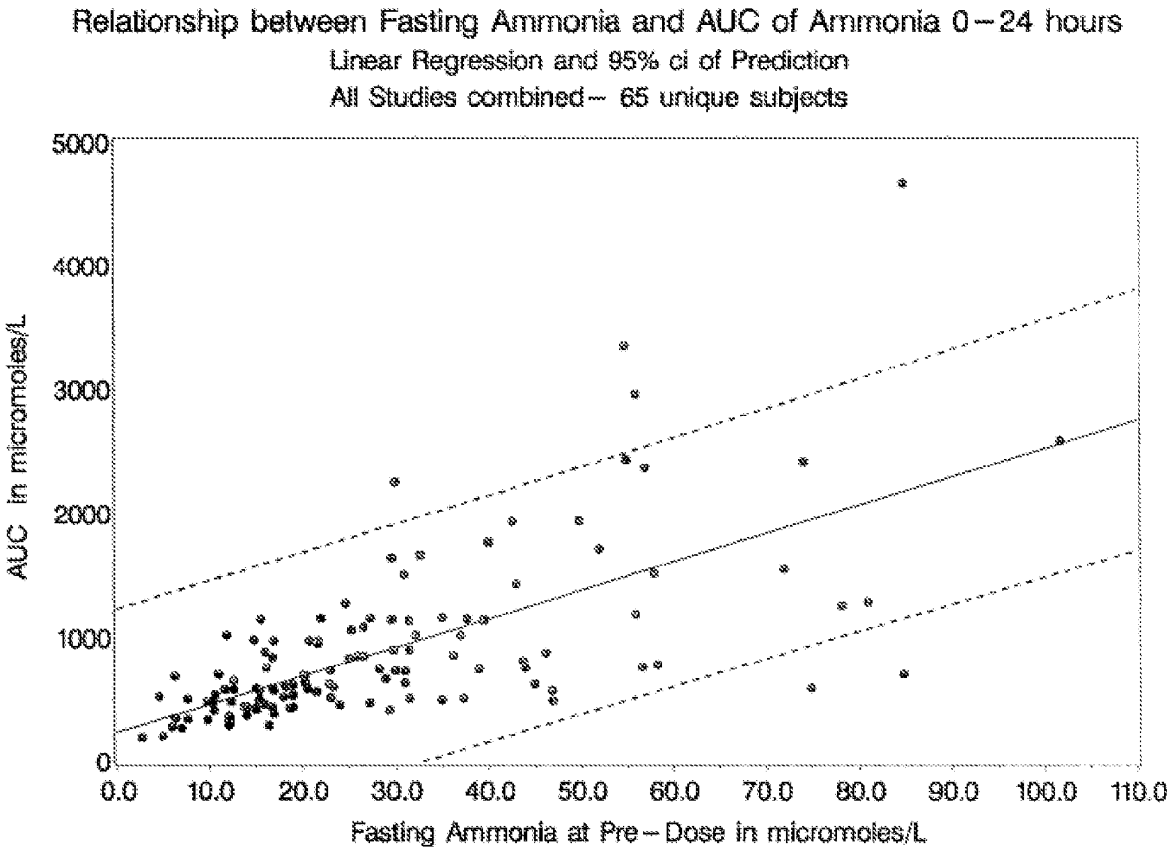

α-Ketoglutarate
NH^+_4
Benzoate
Glycine
NH_4
Glutamate
Glutamine
Phenylacetate
Phenylbutyrate
HCO_3
ATP
Phenylacetylglutamine
Hippurate
CPS
Urine excretion
Carbamyl phosphate
OTC
Citrulline
Aspartate
AS
Ornithine
Urea
Argininosuccinate
Arginase
AL
Arginine
Fumarate
Supplemented arginine

Figure 2


Relationship between Fasting Ammonia and AUC of Ammonia 0 – 24 hours
Linear Regression and 95% ci of Prediction
All Studies combined – 65 unique subjects
AUC in micromoles/L
5000
4000
3000
2000
1000
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0.0
10.0
20.0
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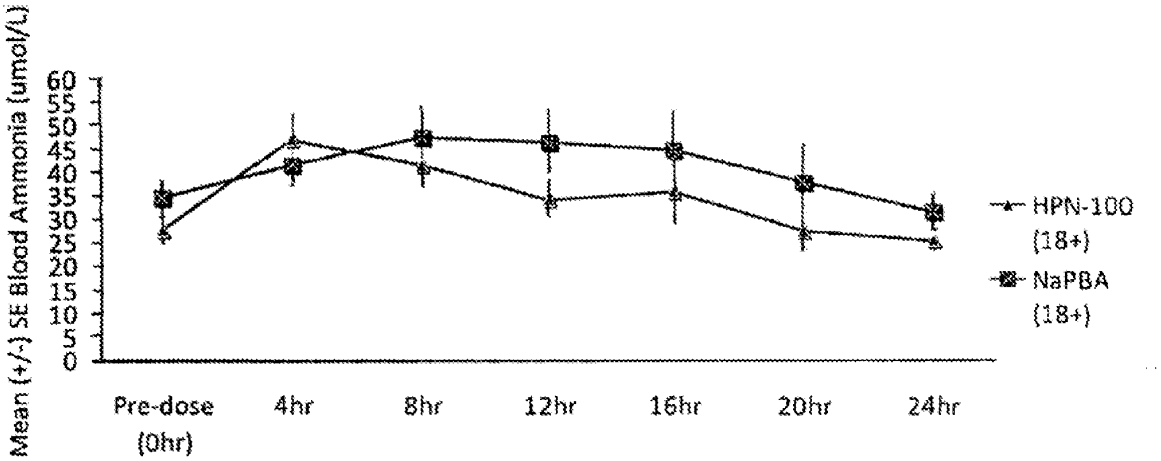
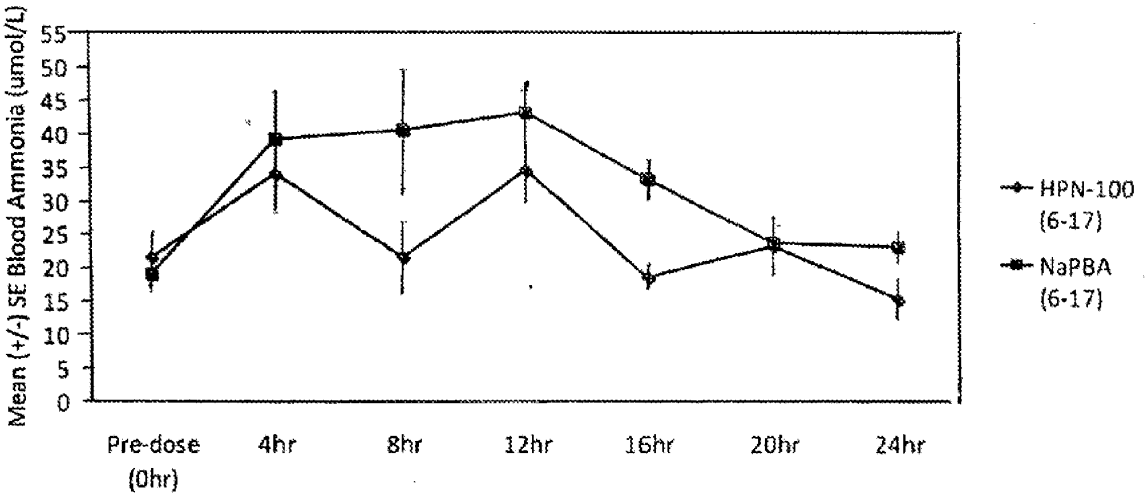
50.0
60.0
70.0
80.0
90.0
100.0
110.0
Fasting Ammonia at Pre – Dose in micromoles/L

Figure 3

A.


Mean (+/-) SE Blood Ammonia (umol/L)
60
55
50
45
40
35
30
25
20
15
10
5
0
Pre-dose (0hr)
4hr
8hr
12hr
16hr
20hr
24hr
HPN-100 (18+)
NaPBA (18+)

B.


Mean (+/-) SE Blood Ammonia (umol/L)
55
50
45
40
35
30
25
20
15
10
5
0
Pre-dose (0hr)
4hr
8hr
12hr
16hr
20hr
24hr
HPN-100 (6-17)
NaPBA (6-17)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: BRUCE SCHARSCHMIDT ET AL.	CONFIRMATION NO.: 7929
APPLICATION NO.: 13/775,000	ART UNIT: 1736
FILING DATE: FEBRUARY 22, 2013	
FOR: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Information Disclosure Statement Within Three Months of Application Filing or Before First Action – 37 C.F.R. § 1.97(b)

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. Timing of Submission

This information disclosure is being filed within three months of the filing date of this application or date of entry into the national stage of an international application or before the mailing date of a first Office action on the merits, whichever occurs last [37 C.F.R. § 1.97(b)]. The references listed on the enclosed Form PTO-1449 (modified) may be material to the examination of this application; the Examiner is requested to make them of record in the application.

2. Cited Information

☐ Copies of the following references are enclosed:

- ☐ All cited references
- ☐ References marked by asterisks
- ☐ The following:

☒ Copies of the following references can be found in related U.S. Application No. 12/350,111:

☒ All cited references
☐ References marked by asterisks
☐ The following:

☒ This application was filed after 30 June 2003 and no copies of U.S. patents nor published applications are enclosed (See Notice of Deputy Commissioner Kunin on 11 July 2003).

☐ The following references are not in English. For each such reference, the undersigned has enclosed (i) a translation of the reference; (ii) a copy of a communication from a foreign patent office or International Searching Authority citing the reference, (iii) a copy of a reference which appears to be an English-language counterpart, or (iv) an English-language abstract for the reference prepared by a third party. Applicant has not verified that the translation, English-language counterpart or third-party abstract is an accurate representation of the teachings of the non-English reference, though, and reserves the right to demonstrate otherwise.

☐ All cited references
☐ References marked by ampersands
☐ The following:

3. Effect of Information Disclosure Statement (37 C.F.R. § 1.97(h))

This Information Disclosure Statement is not to be construed as a representation that: (i) a search has been made; (ii) additional information material to the examination of this application does not exist; (iii) the information, protocols, results and the like reported by third parties are accurate or enabling; or (iv) the cited information is, or is considered to be, material to patentability. In addition, applicant does not admit that any enclosed item of information constitutes prior art to the subject invention and specifically reserves the right to demonstrate that any such reference is not prior art.

4. Fee Payment

No fees are believed due because this Information Disclosure Statement is being filed before the mailing date of the first Office Action.

☐ Applicant further submits that no fee is due in light of the following certification under 37 C.F.R. § 1.97(e) (check only one):

☐ In accordance with 37 C.F.R. § 1.97(e)(1), the undersigned hereby states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart

foreign application not more than three months prior to the filing of this statement; or

☐ In accordance with 37 C.F.R. § 1.97(e)(2), the undersigned hereby states that no item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart foreign application, or, to the knowledge of the person signing the certification after making reasonable inquiry, was known to any individual designated in 37 C.F.R. § 1.56(c), more than three months prior to the filing of this statement.

However, should the Commissioner determine that fees are due in order for this Information Disclosure Statement to be considered, the Commissioner is hereby authorized to charge such fees to Deposit Account No. 50-2586.

5. Patent Term Adjustment (37 C.F.R. § 1.704(d))

☐ The undersigned states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart application and that this communication was not received by any individual designated in 37 C.F.R. § 1.56(c) more than thirty days prior to the filing of this statement. 37 C.F.R. § 1.704(d).

Respectfully submitted,
Perkins Coie LLP

Date: April 18, 2013

/
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT Form PTO-1449 (Modified) (Use several sheets if necessary)				COMPLETE IF KNOWN	
				Application Number	13/775,000
				Confirmation Number	7929
				Filing Date	2013-02-22
				First Named Inventor	Bruce SCHARSCHMIDT
				Group Art Unit	1736
				Examiner Name	To be assigned
Sheet	1	of	10	Attorney Docket No.	79532.8003.US03

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	U.S. Patent or Application NUMBER	Kind Code (if known)	Name of Patentee or Inventor of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Figures Appear
	A1	2004/0229948	A1	SUMMAR et al.	11/18/2004	
	A2	2006/0135612	A1	FERRANTE	06/22/2006	
	A3	2008/119554	A1	JALAN et al.	05/22/2008	
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	A5	5,968,979		BRUSILOW	10/19/1999	
	A6	6,050,510	A	BONNEWITZ	05/09/2000	
	A7	6,083,984		BRUSILOW	07/04/2000	

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Examiner Initial	Cite No.	Foreign Patent or Application Office	NUMBER	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Figures Appear	T
	B1	WO	2005/053607	A1	MEDICIS PHARMACEUTICAL CORP.	06/16/2005		
	B2	WO	2006/056794		UCL BUSINESS PLC	06/01/2006		
	B3	WO	2009/087474		AKTHELIA PHARMACEUTICALS	07/16/2009		
	B4	WO	2009/134460	A1	HYPERION THERAPEUTICS	11/05/2009		
	B5	WO	2010/0250303	A1	HYPERION THERAPEUTICS	03/04/2010		

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C1	AMBROSE, A.M. et al. (1933). "Further Studies on the Detoxification of Phenylacetic Acid," J. Bio. Chem. 101:669-675.	
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	C3	BATSHAW, M.L. et al. (August 1981). "New Approaches to the Diagnosis and Treatment of Inborn Errors of Urea Synthesis," Pediatrics 68(2):290-297.	
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	C21	ClinicalTrials.Gov/Archive View of NCT00551200 on 2007_12_11 "Dose-Escalation Safety Study of Glyceryl Tri (4-Phenylbutyrate)(GT4P) to Treat Urea Cycle Disorders" [accessed 5 October 2009], 4 pages.	
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	C23	Combined Search and Examination Report mailed on October 9, 2009, for Great Britain Patent Application No. GB0915545.8, filed on August 27, 2009, eight pages.	
	C24	COMTE, B., et al., "Identification of Phenylbutyrylglutamine, A new Metabolite of Phenylbutyrate Metabolism in Humans," Journal of Mass Spectrometry (2002) 37(6):581-590.	
	C25	DEFERRARI, G. et al. (1981). "Brain Metabolism of Amino Acids and Ammonia in Patients with Chronic Renal Insufficiency," Kidney International 20:505-510.	
	C26	DIAZ, G.A., et al., "Phase 3 Blinded, Randomized, Crossover Comparison of Sodium Phenylbutyrate (NaPBA) and Glycerol Phenylbutyrate (GPB): Ammonia (NH3) Control in Adults with Urea Cycle Disorders (UCDs)," Mol. Genet. Metab. 102:276 (2011).	
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	C29	Examination Report mailed May 11, 2010, for United Kingdom Patent Application No. GB0915545.8, filed on August 27,2009, one page.	
	C30	FDA. (August 2003). "Buphenyl® (Sodium Phenylbutyrate) Label" nine pages.	
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	C40	International Preliminary Report on Patentability mailed on March 1, 2011, for PCT Application No. PCT/US2009/030362, filed on January 7, 2009, seven pages.	
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	C43	JOHN, BA et al. (March 2009). "The Disposition of HPN-100, A Novel Pharmaceutical Under Development for Potential Treatment of Hyperammonemia, in Cynomologus Monkeys," abstract presented at ACMG 2009, one page.	
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	C50	LEE, B. et al. (August 2008). "Preliminary Data on Adult Patients with Urea Cycle Disorders (UCD) in an Open-Label, Switch-Over, Dose-Escalation Study Comparing a New Ammonia Scavenger, Glyceryl Tri (4-Phenylbutyrate) [HPN -100], to Buphenyl® (Sodium Phenylbutyrate [PBAj)," abstract presented at SSIEM 2008, Lisbon, Portugal, one page.	
	C51	LEE, B. et al. (September 2008). "Preliminary Data on Adult Patients with Urea Cycle Disorders (UCD) in An Open-Label, Switch-Over, Dose Escalation Study Comparing A New Ammonia Scavenger, Glyceryl Tri (4-Phenylbutyrate) [HPN-1 00], to BUPHENYL® (Sodium Phenylbutyrate [PBA]," presented at SSIEM 2008, Lisbon, Portugal, Poster, one page.	
	C52	LEE, B., et al.,"Phase 2 Comparison of a Novel Ammonia Scavenging Agent with Sodium Phenylbutyrate in Patients with Urea Cycle Disorders: Safety, Pharmacokinetics and Ammonia Control," Mol. Genet. Metab. 100:221-228 (2010).	
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OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS			
Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C58	MANSOUR, A. et al. (October 1997). "Abdominal Operations in Patients with Cirrhosis: Still A Major Surgical Challenge," Surqerv 122(4):730-735. (Abstract Only.)	
	C59	MASETRI, N.E. et al. (August 1992). "Plasma Glutamine Concentration: A Guide in the Management of Urea Cycle Disorders," J. Pediatr. 121 (2):259-261.	
	C60	MCGUIRE, B. M., et al., "Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis," Hepatol. 51:2077-2085 (2010).	
	C61	MCGUIRE, B.M. et al. (2009). "Pharmacokinetic (PK) and Safety Analyses of a Novel Ammonia-Reducing Agent in Healthy Adults and Patients with Cirrhosis," Hyperion Therapeutics, poster, one page.	
	C62	MCGUIRE, B.M. et al. (May 2009). "Pharmacokinetic (PK) and Safety Analyses of a Novel Ammonia-Reducing Agent in Healthy Adults and Patients with Cirrhosis," abstract presented at DDW, May 2009, two pages.	
	C63	MCGUIRE, B. et al. (April 2008). "Pharmacokinetic Safety Study of Sodium Phenylacetate and Sodium Benzoate Administered to Subjects With Hepatic Impairments,' Liver International 28:743. (Abstract Only).	
	C64	MCGUIRE, B. et al. (April 2008). "Pharmacokeinetic (PK) Safety Study of Sodium Phenylacetate and Sodium Benzoate Administered to Subjects with Hepatic Impairment," abstract of The13th International Symposium, Abano (Padova), Italy, April 28-May 1,2008, two pages.	
	C65	MCQUADE P.S. (1984). "Analysis and the Effects of Some Drugs on the Metabolism of Phenylethylamine and Phenylacetic Acid," Neuropsychopharmacol. Bioi. Psychiat. 8:607-614.	
	C66	PISCITELLI, S.C. et al. (1995). "Disposition of Phenyl butyrate and its Metabolites, Phenylacetete and Phenylacetylglutamine," J. Clin. Pharmacal. 35:368-373.	
	C67	PROPST, A. et al. (August 1995). "Prognosis and Life Expectancy in Chronic Liver Disease," Dig Dis Sci 40(8):1805-1815. (Abstract Only).	

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

INFORMATION DISCLOSURE STATEMENT BY APPLICANT Form PTO-1449 (Modified) (Use several sheets if necessary)				COMPLETE IF KNOWN	
				Application Number	13/775,000
				Confirmation Number	7929
				Filing Date	2013-02-22
				First Named Inventor	Bruce SCHARSCHMIDT
				Group Art Unit	1736
				Examiner Name	To be assigned
Sheet	9	of	10	Attorney Docket No.	79532.8003.US03

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS			
Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C68	RILEY, T.R. et al. (November 15, 2001). "Preventive Strategies in Chronic Liver Disease: Part II. Cirrhosos," Am. Fam. Physician 64(10):1735-1740. (Abstract Only).	
	C69	RUDMAN, D., et al., "Maximal Rates of Excretion and Synthesis of Urea in Normal and Cirrhotic Subjects," J. Clin. Invest. (1973) 52:2241-2249.	
	C70	SHIPLE, G.J. et al. (1922). "Synthesis of Amino Acids in Animal Organisms. I. Synthesis of Glycocoll and Glutamine in the Human Organism," J. Am. Chem. Soc. 44:618-624.	
	C71	SIMELL, O., et al., "Waste Nitrogen Excretion Via Amino Acid Acylation: Benzoate and Phenylacetate in Lysinuric Protein Intolerance," Pediatr. Res. 20(11):1117-1121 (1986).	
	C72	SINGH, "Consensus Statement from a Conference for the Management of Patients with Urea Cycle Disorders," Suppl. to J. Pediatrics (2001) 138(1):S1-S5.	
	C73	SUMMAR, M.L. et al. (October 2008, e-pub. July 17, 2008). "Diagnosis, Symptoms, Frequency and Mortality of 260 Patients with Urea Cycle Disorders From a 21-Year, Multicentre Study of Acute Hyperammonaemic Episodes," Acta Paediatr. 97:1420-1425.	
	C74	SUMMAR, M. et al. (2007). "Description and Outcomes of 316 Urea Cycle Patients From a 21-Year, Multicenter Study of Acute Hyperammonemic Episodes," Abstract, presented at Annual Symposium CCH - Congress Centre Hamburg, September 4-7,2007, GSSIEM 2007, two pages.	
	C75	SWEDISH ORPHAN INTERNATIONAL. (January 12, 2007). "Urea Cycle Disorders an International Perspective," Poster, Symposium Swedish Orphan International, Barcelona, Spain, January 12, 2007, one page.	
	C76	TANNER, L. M., et al., "Nutrient Intake in Lysinuric Protein Intolerance," J. Inherit. Metab. Dis. 30:716-721 (2007).	
	C77	THIBAULT, A., et al., "A Phase I and Pharmacokinetic Study of Intravenous Phenylacetate in Patients with Cancer," Cancer Res. 54:1690-1694 (1994).	

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				First Named Inventor	Bruce SCHARSCHMIDT
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Sheet	10	of	10	Attorney Docket No.	79532.8003.US03

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Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C78	THIBAULT, A., et al., "Phase I Study of Phenylacetate Administered Twice Daily to Patients with Cancer," Cancer 75:2932-2938 (1995).	
	C79	TUCHMAN, M. et al. (2008, e-pub. June 17, 2008). "Cross-Sectional Multicenter Study of Patients With Urea Cycle Disorders in the United States," Malec. Genetics Metab. 94:397-402.	
	C80	WATERLOW, J.C. (March 1963). "The Partition of Nitrogen in the Urine of Malnourished Jamaican Infants," Am. J. of Clin. Nutrition 12:235-240.	
	C81	ZEITLIN, P.L. et al. (July 2002). "Evidence of CFTR Function in Cystic Fibrosis After System Administration of 4-Phenylbutyrate," Mol. Therapy 6(1):119-126.	

EXAMINER	DATE CONSIDERED

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Electronic Acknowledgement Receipt	
EFS ID:	15558863
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Lara J. Dueppen/Colleen Kirchner
Filer Authorized By:	Lara J. Dueppen
Attorney Docket Number:	79532.8003.US03
Receipt Date:	18-APR-2013
Filing Date:	22-FEB-2013
Time Stamp:	18:23:45
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	Response.pdf	48577 4b848e70f95f82cbf1712fff15a621853074a52f	no	1
Warnings:					
Information:					

2	Oath or Declaration filed	8003US03_Declarations.pdf	114679 eee723da76e411208cbf9ae201157eeaf2c79622	no	2
Warnings:					
Information:					
3	Drawings-only black and white line drawings	Replacement_Figures.pdf	462513 bae0cca8e79b9fbe87ba69be12e5f165dacbac9d	no	3
Warnings:					
Information:					
4	Transmittal Letter	IDS_transmittal.pdf	104559 c9ec7f6a3c34c66662391 7f5bc7b7df0635c1286	no	3
Warnings:					
Information:					
5	Information Disclosure Statement (IDS) Form (SB08)	IDS_Form.pdf	140694 f57bf27f09b2168734af4f7d8168bbabdce29bdd	no	10
Warnings:					
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<u>New International Application Filed with the USPTO as a Receiving Office</u>
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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/775,000

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	x 40 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				390			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1120		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/775,000	02/22/2013	1736	828	79532.8003.US03	11	3

CONFIRMATION NO. 7929

UPDATED FILING RECEIPT

34055
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POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

OC000000060646598

Date Mailed: 04/29/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

Applicant(s)

HYPERION THERAPEUTICS, INC., South San Francisco, CA

Assignment For Published Patent Application

HYPERION THERAPEUTICS, INC., South San Francisco, CA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a DIV of 13/417,137 03/09/2012 PAT 8404215 *
which claims benefit of 61/542,100 09/30/2011
and claims benefit of 61/564,668 11/29/2011
(*)Data provided by applicant is not consistent with PTO records.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/775,000**

Projected Publication Date: Perfected

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Preliminary Class

423

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/775,000	02/22/2013	1629	828	79532.8003.US03	11	3

CONFIRMATION NO. 7929

UPDATED FILING RECEIPT

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

Date Mailed: 05/06/2013

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

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Masoud Mokhtarani, Walnut Creek, CA;

Applicant(s)

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Assignment For Published Patent Application

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Power of Attorney: None

Domestic Priority data as claimed by applicant

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Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
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Projected Publication Date: 08/15/2013

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****
Title

METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Preliminary Class

424

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/775,000

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	70		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	300		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	360		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	20 minus 20 =	*	x 40 =	0.00	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*	x 210 =	0.00			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				390			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1120		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
Application Size Fee (37 CFR 1.16(s))									
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/775,000	02/22/2013	Bruce Scharschmidt	79532.8003.US03

CONFIRMATION NO. 7929

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SEATTLE, WA 98111-1247

PUBLICATION NOTICE

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Publication No.US-2013-0210914-A1
Publication Date:08/15/2013

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The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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				Application Number	13/775,000
				Confirmation Number	7929
				Filing Date	February 22, 2013
				First Named Inventor	SCHARSCHMIDT, Bruce
				Group Art Unit	1736
				Examiner Name	
Sheet	1	of	3	Attorney Docket No.	79532.8003.US03

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	U.S. Patent or Application NUMBER	Kind Code (if known)	Name of Patentee or Inventor of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	6,219,567		EGGERS	4/17/2001	
	A2	8,642,012		SCHARSCHMIDT	2/4/2014	
	A3	2010/0008859		SCHARSCHMIDT	1/14/2010	
	A4	2012/0022157		SCHARSCHMIDT		
	A5	2012/0220661		LEE		
	A6	2013/0210914		SCHARSCHMIDT		

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.	Foreign Patent or Application Office	NUMBER	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	B1	WO	2007/005633					
	B2	WO	2009/087474		Akthelia Pharmaceuticals	7/16/2009		
	B3	WO	2012/028620					

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C1	BATSHAW, M.L. et al. (1981, August) "New Approaches to the Diagnosis and Treatment of Inborn Errors of Urea Synthesis," *Pediatrics* 68(2):290-297.	
	C2	BRAHE, C., et al., (2005) "Phenylbutyrate Increases SMN Gene Expression in Spinal Muscular Atrophy Patients," *Eur J Hum Genet* 13:256-259.	
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EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

79532-8003.US03/LEGAL124080099.1

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OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C6	DIAZ, G.A.. et al.. "Phase 3 Blinded. Randomized, Crossover Comparison of Sodium Phenylbutyrate (NaPBA) and Glycerol Phenylbutyrate (GPB): Ammonia (NH3) Control in Adults with Urea Cycle Disorders (UCDs)," *Mol. Genet. Metab.* 102:276, *Society of Inherited Metabolic Disease* (SMID) Abstract.	
	C7	ENNS, G.M., et al., (2007) "Survival After Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders," *N Eng J Med* 356:2282-2292.	
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	C12	HYPERION THERAPEUTICS "Hyperion Therapeutics Announces Enrollment of First Patient in Phase 1/2 Clinical Trial of GT4P in Patients with Urea Cycle Disorders" Announcement, 1 page (October 23, 2007).	
	C13	MERCURI, E., et al., (2004) "Pilot Trial of Phenylbutyrate in Spinal Muscular Atrophy," *Neuromuscul Disord* 14:130-135.	
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	C18	PERRINE, S. P., (2008) "Fetal Globin Stimulant Therapies in the Beta-Hemoglobinopathies: Principles and Current Potential," *Pediatr Ann* 37(5):339-346.	
	C19	RYU, H., et al., (2005) "Sodium Phenylbutyrate Prolongs Survival and Regulates Expression of Anti-Apoptotic Genes in Transgenic Amyotrophic Lateral Sclerosis Mice," *J Neurochem* 93:1087-1098.	
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	C22	EUROPEAN PATENT OFFICE, Extended European Search Report for EP09739263 completed November 2, 2011.	
	C23	EUROPEAN PATENT OFFICE, International Search Report and Written Opinion for PCT/US2009/055256 completed December 18, 2009 and mailed December 30, 2009.	
	C24	Examination Report for British Patent Application No. GB1013468.2 dated October 28, 2011.	
	C25	International Preliminary Report on Patentability (Ch I) for PCT/US2012/028620 completed June 4, 2012 and mailed on April 10, 2014.	
	C26	International Preliminary Report on Patentability (Ch II) for PCT/US2012/028620, completed August 22, 2013 and mailed September 4, 2013.	
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	C28	UNITED STATES PATENT AND TRADEMARK OFFICE, International Search Report and Written Opinion for PCT/US2012/028620 mailed June 20, 2012.	
	C29	UNITED STATES PATENT AND TRADEMARK OFFICE, International Search Report and Written Opinion for PCT/US2012/54673 mailed November 20, 2012.	
	C30	UNITED STATES PATENT AND TRADEMARK OFFICE, International Search Report and Written Opinion for PCT/US2013/71333 mailed March 28, 2014.	

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(74) Agent: **RUBEN, Bradley, Noell**; 463 1st Street, Suite 5a, Hoboken, NJ 07030-1859 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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WO 2007/005633 A2

(54) Title: PROCESS FOR PREPARATION OF LIQUID DOSAGE FORM CONTAINING SODIUM 4-PHENYLBUTYRATE

(57) Abstract: A process for preparing a stable aqueous dosage form of sodium 4-phenylbutyrate, including such dosage forms in a highly concentrated solution, as well as methods for making 4-phenylbutyrate and 4-phenylbutyric acid, and for using 4-phenylbutyrate. The stable aqueous dosage forms do not freeze at 0° C.

Process for preparation of liquid dosage form containing sodium 4-phenylbutyrate

Field of the Invention

This invention relates to a process of preparing a highly concentrated solution of sodium 4-phenylbutyrate in an aqueous medium useful as an alternative for present high dosage therapeutic treatments of urea cycle deficiencies, sickle-cell anemia, and cancer.

Back ground of the Invention

Sodium 4-phenylbutyrate is currently being prescribed to treat urea cycle deficiency in children; it is sold in the USA under the trademark BUPHENYL (Ucyclyd Pharma, Inc., Glen Burnie, MD), and in Europe under the trademark AMMONAPS (Orphan Europe). The urea cycle is the metabolic process by which the human body gets rid of nitrogen. There are six enzymes that take part in this process. A deficiency of any one of them upsets the process and causes excess nitrogen, in the form of ammonia, to accumulate in the body. The six urea cycle disorders are: carbamyl phosphate synthetase deficiency; n-acetylglutamate synthetase deficiency; ornithine transcarbamylase deficiency (the most common type); argininosuccinic acid synthetase deficiency (also called citrullinemia); argininosuccinase acid lyase deficiency; and arginase deficiency. Nitrogen accumulation is also present in patients with kidney or liver failure.

In children born with any of these rare enzyme deficiencies in the urea cycle, if the enzyme deficiency is severe, the condition leads to coma and death within a few days of birth. Such children are unable to excrete waste nitrogen as urea. Accordingly, the waste nitrogen accumulates as ammonium ions in the plasma leading to a condition known as hyperammonemia. Such genetic defects cannot be cured, but the condition can be treated by adherence to a life-long combination of a low protein diet and the administration of suitable medication. Presently, a combination of sodium phenylacetate and sodium benzoate is administered to children who have an N-acetylglutamine synthetase-1 deficiency, whereas sodium 4-phenylbutyrate (typically in a dosage of 450-600 mg/kg/day in three or more divided doses) is administered to children having an ornithine transcarbamoylase deficiency. In the

latter treatment, the sodium 4-phenylbutyrate is converted to 2-phenylacetate, which combines with the amino acid glutamine present in the plasma and the resulting combination (or conjugate) is excreted as phenylacetylglutamine in the urine. Thus, administration of sodium 4-phenylbutyrate provides an alternative to the urea pathway as a means of excreting waste nitrogen from the body.

The above-mentioned commercially available forms of 4-phenylbutyrate, BUPHENYL in the US and AMMONAPS in Europe, are marketed as a granular powder for making a solution for oral administration to infants and young children, and as 500 mg tablets for adults and children weighing over 20 kg. The powder dosage is measured in one of three differently sized measuring spoons, which always leads to an imprecise dosage level. For example, a six year old child suffering from ornithine transcarbamoylase deficiency and weighing19 kg has to take 3.8 g of powdered sodium 4-phenylbutyrate three times daily. The imprecise dosing measurement, and the need to mix the powder with a fluid for administration, leads to a lack of compliance in taking the prescribed dose at the required intervals. Consequently, it is invariably the case that children have to be admitted to hospital, sometime two or three times a year, because they feel nauseous, this being a first sign of hyperammonaemia caused by failure to maintain the dosing regimen. The symptom of nausea means the child patient cannot take the powder orally. Accordingly, in hospital the patient is treated with an intravenous infusion of sodium 4-phenylbutyrate (or sodium phenylacetate and sodium benzoate) to reduce the ammonium ion level to normal. When the nausea subsides, normal oral therapy is then resumed. Unfortunately, sometimes the delay in reaching a hospital leads to the patient being admitted in a hyperammonaemic coma; death may result or, on recovery, the child may be permanently brain-damaged.

Another important requirement for high dosage medications such as sodium 4-phenylbutyrate is the purity. High dosages such as 4 g per day or more require the purest of starting materials and good process control to bring all the impurities to less than 0.05% w/w.

WO 85/04805 discloses a process for waste nitrogen removal in human beings, wherein a compound having the formula $Ph\text{-}CH_2\text{-}(CH_2)_n\text{-}COOH$, wherein n is 2, such as 4-phenylbutyrate, is administered.

US Pat. App. 2004/0180962 discloses a delayed release methodology for using a low dosage of sodium 4-phenylbutyrate to treat urea cycle deficiency by

compounding in a tablet form with hydroxypropylmethylcellulose and a release-controlling excipient (a release retarder or a liberation controller). However, such delayed release methodologies are not the best approach for treating this particular disease because a sufficient amount of the metabolite (phenylacetate) must be present in the plasma to react with glutamine and then be excreted as phenylacetylglutamine.

US Pat. App. 2004/0152784 describes a pharmaceutical composition of sodium 4-phenylbutyrate with effective aromatic flavoring agent and at least one synthetic sweetening agent. This disclosure provides a dry granulated pharmaceutical composition that can be dissolved in water before administration. One of the examples provides a maximum concentration of sodium 4-phenylbutyrate in the reconstituted solution of 250 mg/mL at 10 °C. This reconstituted solution would require a relatively a large volume of solution for a suitable dosage, making it difficult to administer the drug to infants because of the large liquid volumes necessary upon dissolving the granules in water. Also, this particular pharmaceutical preparation is not stable biologically as it does not contain any preservative.

The '784 application also demonstrates that the sweetening agent (potassium aspartame) is not stable in the aqueous reconstituted solution of the dry powder containing sodium 4-phenylbutyrate because it loses its sweetness when stored for more than a few weeks. The drug 4-phenylbutyrate is a very bitter-tasting compound, so loss of sweetness leads to a lack of compliance with the dosing regimen. Accordingly, additional precautions are needed when using the formulation is the '784 application.

Sodium 4-phenylbutyrate is also useful for treating a variety of other medical indications, such as benign prostate hyperplasy, certain cancers, cystic fibrosis; HIV, spinocerebellar ataxia, kidney and liver failures, and thalasemia.

Another use for sodium phenylbutyrate is to induce fetal hemoglobin production in patients with sickle cell anemia; this has been described by George J. Dover (*Blood,* vol. 84, No. 1, Jul. 1, 1994: pp 339-343). This paper states that sodium phenylbutyrate in powdered form has a bitter taste that, despite many attempts, cannot be disguised. Two of the four subjects treated as outpatients reported an inability to maintain compliance with their dosing regimen because of the high dosage requirements (30 to 40 tablets per day).

DE 19,810,383 describes 4-phenylbutyrate as an apoptosis-inducing agent for neoplastic therapy.

WO 9937150 describes a transcription therapy for cancer using a retinoic acid and/or an inhibitor of histone deacetylase. For this treatment, 4-phenylbutyrate is classified as a histone deacetylase inhibitor.

WO 93/07866, WO 9510271, and EP 725635 all disclose compositions and methods using phenylacetic acid (a metabolite of 4-phenylbutyrate) and its derivatives for therapy and prevention of a number of pathologies, Including cancer, AIDS, anemia, and severe beta-chain hemoglobinopathies. A number of U.S. patents describe the use of phenylacetic acid as an anticancer agent (*e.g.*, 6,037,376) and as an anti-viral agent (*e.g.*, 5,877,213 and 5,710,178).

WO 9856370 and US 6,207,195 describe therapeutic sodium 4-phenylbutyrate containing nanospheres for the treatment of cystic fibrosis by CFTR gene therapy.

US Pat. App. 2003/0195255 describes a method of administering sodium 4-phenylbutyrate orally to treat loss of mental function associated with chronic hepatic encephalopathies, recommending a high dosage of about 200-300 mg//kg initially over one to two hours, and then divided into three equal dosages daily; for adults the dose is described as 3 to 12 g/m^2. With regard to the synthetic of sodium 4-phenylbutyrate and related compounds, some of the methods involve using substituted malonic esters.

WO 9901420 and WO 9503271 each describes a process of preparing substituted amino malonic acid and α-amino substituted propanoic acid from its ethyl ester. Preparation of substituted butyric acid from substituted malonic esters using various reagents is reported in several published research papers. *J. Med. Chem.*, 47 (12), 3282-3294, 2004; *Bioorg & Med Chem.*, 11(1), 113 -121, 2003; *J. Med. Chem.*, 46 (10), 2008-2016, 2003; *Enantiomer*, 7(1), 1-3, 2002; *J. Med. Chem.*, 45 (2), 263-274, 2002; *J. Het. Chem.*, 25(6), 1689–1695, 1988.

In addition, 4-phenylbutyrate has been shown as useful for protecting againt cerebral ischemic injury. (X. Qi, *et al.*, *Mol. Pharmacol.*, 66(4), 899-908 (2004).)

Commercial manufacturing of 4-phenylbutyric acid involves the potential carcinogen benzene as one of the raw materials. US Pat. No. 6,372,938 Burzynski *et al.; J. Am. Chem. Soc.*, 74, 1591 (1952); *J. Am. Chem. Soc.* 74; 4721 (1952); *Bull.Acad.Sci.USSR Div.Chem.Sci.*(Engl.Transl.), EN, 36, 2, 327-330 (1987); *Akad. Nauk SSSR Ser. Khim.*; RU, 2; 367-371 (1987).

Summary and Objects of the Invention

Sodium 4-phenylbutyrate is a very bitter-tasting compound and so it is very difficult for patients to comply with their dosing regimen, especially children who have to take large amounts of the medicine every day. It would be of immense benefit to the children and their parents if the oral dosage were more palatable, easier to administer, and/or have a lower volume liquid dosage form, and preferably a combination of all three. The treatment works, but non-compliance with the present dosing regimen causes incomplete treatment leading to occasional hospitalization.

Accordingly, one object of this invention is to provide an improved pharmaceutical composition containing sodium 4-phenylbutyrate for the use by patients presently administered with a high dosage and high volume dose of this drug. To accomplish this, one embodiment of this invention provides a process for preparing a liquid dosage of sodium 4-phenylbutyrate in a more concentrated aqueous solution than provided by the present art, preferably containing at least one of a preservative and a sweetening agent, and preferably both, in addition to a flavoring agent; a fragrance can also be added. The supersaturated solution can have a concentration up to 500 mg/mL of sodium 4-phenylbutyrate or more; preferably the concentration ranges from about 300 mg/mL to about 700 mg/mL. A preservative such as sodium benzoate can be present, preferably at about 2.5 mg/mL. In other embodiments, the dosage can include a sweetening and/or other flavoring agent, such as about 2 mg/mL of sodium saccharine, 0.01 mg/mL of sucralose, and/or about 2 mg/mL of raspberry flavoring. This highly concentrated liquid dosage is more concentrated and more palatable, leading to easier administration to young patients and facilitating improved compliance to the dosing regimen. This concentrated solution is effective and very easy to administer to babies because it requires only a few milliliters at any one dosing time; and it is easy to administer to children because each dosage is only a few milliliters of solution at any one time.

In another embodiment, this invention provides a process of preparing a supersaturated solution of sodium 4-phenylbutyrate in water by adding sufficient water to a known quantity of sodium 4-phenylbutyrate at an elevated temperature of about 30° to about 80° C to produce a concentration of about 600 mg/mL.

Yet another object of this invention is to provide a process for manufacturing sodium 4-phenylbutyrate with impurities at a level less than 0.05% (weight/weight

basis). The general process provided by this invention is to treat $Ph\text{-}(CH_2)_2\text{-}CH(COOEt)_2$ (*i.e.,* diethyl 2-phenylethylmalonate) with acetic acid and aqueous hydrochloric acid to produce 4-phenylbutyric (or 4-phenylbutanoic) acid. In another and continuing embodiment, conversion of 4-phenylbutyric acid to its sodium salt is accomplished in an organic solvent medium with an inorganic base.

The present invention is a novel method of synthesis of 4-phenyl butyrate without benzene.

In summary this invention provides a pharmaceutical liquid composition, comprising a solution of sodium 4-phenylbutyrate in an aqueous medium at a concentration of at least about 300 mg//mL, including generally at a concentration of 300 mg/mL to about 700 mg/mL, and more preferably at a concentration of 400 mg/mL to about 600 mg/mL. As a dosage the composition preferably further comprises at least one or more of a flavoring agent, including sweeteners, a preservative, and compatible mixtures thereof. The composition may also include an inorganic base.

This invention also provides a process for making a highly concentration solution of 4-phenylbutyrate by dissolving the same in water, preferably at an elevated temperature.

This invention also provides a process for making 4-phenylbutyrate from 4-phenylbutyric acid by dissolving the same in an organic medium, treating with an inorganic alkali, heating, adding a second solvent to precipitate the product, and isolating/purifying the product.

This invention also provides a process for making 4-phenylbutyric acid from a diester of the formula $Ph\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}(COOR)_2$ wherein R is an alkyl of not more than four carbons, aryl, or aralkyl wherein the alkyl portion has not more than four carbons, treating the same with a mineral acid, precipitating the product, and thereafter isolating and/or purifying the same.

This invention also provides a method of treating a patient suffering from a urea cycle deficiencies, sickle-cell anemia, cancer, or potential cerebral ischemic injury, comprising providing an oral aqueous solution of 4-phenylbutyrate having a concentration of at least about 300 mg/mL and orally administering said solution to a patient in need thereof.

Detailed description of the specific embodiments

This invention relates to an oral liquid pharmaceutical multiple dosage form of sodium 4-phenylbutyrate in a supersaturated solution in an aqueous medium, preferably containing at least one preservative. The drug concentration in the formulation is achieved to a maximum of about 700 mg/mL, and at 600 mg/mL the solution does not freeze at 0° C.

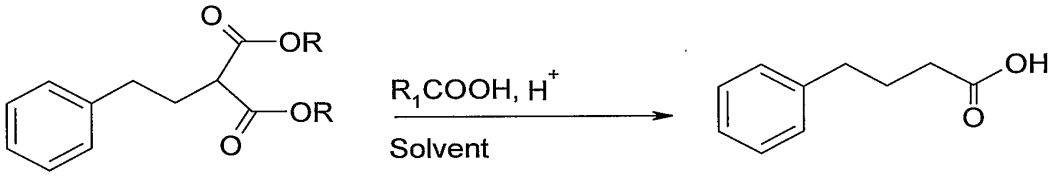
Thermodynamically, the solubility of a species is dependent upon temperature and the interaction between the species and the solvent through various types of intermolecular and intramolecular interactions. The solute--solvent intermolecular interactions are the prime reason for the change in solubility at different temperatures. For a true solution, at a relatively higher temperature the solute–solvent intermolecular interaction is more pronounced than at a relatively lower temperature, and thus it is typically observed the solubility of a compound soluble in a given solvent increases as the temperate increases.

In this invention it has been found that the solubility of sodium 4-phenylbutyrate has been found to be exceptionally higher than that reported in the prior art (for example, the above-mentioned US application 2004/0152784 reports a maximum solubility of sodium 4-phenylbutyrate of 250 mg/mL at 10° C.). This art-reported solubility is believed to pertain to the maximum solubility of the monomeric form of sodium 4-phenylbutyrate in water.

As described in more detail below, this invention describes a process of preparing a highly concentrated solution of sodium 4-phenylbutyrate, having a concentration 500 mg /mL in water by dissolving 5 g of sodium 4-phenylbutyrate in about 3.5 mL water to yield a solution volume of about 10 mL. The temperature can be room temperature (25° C) or an elevated temperature, preferably in the range of up to about 80° C. We found it is more difficult to make this solution at room temperature, but the solution can be made at a higher temperature and then cooled to room temperature without precipitating resulting. The solution thus made was believed to be a supersaturated, non-ideal solution that does not obey the van't Hoff equation (a plot of –ln K versus 1/T giving a straight line, where K is the solubility constant and T is the absolute temperature). While not desirous of being constrained to a particular theory, these results suggest to us that that the solution so formed is a micellar kinetic phase where sodium 4-phenyl butyrate is the micelle in an aqueous bulk phase. Therefore, due to likely micelle formation of sodium 4-phenylbutyrate (which we term the self-associated polymeric form), the high concentration of about 500 mg/mL can

be achieved in solution. Even further, this high concentration solution did not freeze or precipitate out upon storage, even at 0 °C for two days, and only on further cooling to -4° C is precipitation observed. This novel invention thus provides a dosage form better able to help the patients presently administered with a high volume dosage of sodium 4-phenylbutyrate. This invention is not intended to be limited by this discussion of micellar phases, or the presence or absence of other high concentration phases (such as sponge or L3, worm-like micelles, sheets and other laminar phases) that may be formed depending on the particular processing conditions and/or materials used. In the follow description the term "solution" is used without regard to whether a micellar phase is present.

In another embodiment this invention provides a process for preparing 4-phenylbutyric acid by the scheme shown below, where an organic ester is treated with an acid in a solvent, optionally concentrating the product, purifying the product, and optionally further purifying the product.



R_1 = Methyl, Ethyl, Propyl, Chloromethyl, Bromomethyl,
R = Methyl, Ethyl

In this process, an organic ester of the formula Ph-CH_2-CH_2-CH-$(COOR)_2$ is treated with a mineral acid in a water miscible organic solvent medium at a desired temperature. Each R is independently an alkyl group containing up to four carbon atoms, or an aryl or aralkyl group wherein the alkyl portion has up to four carbon atoms. The resulting product may be concentrated, such as by evaporation (vacuum and/or temperature induced). Thereafter, the product 4-phenylbutyric acid is precipitated from solution with the aid of a non-polar solvent. This crude 4-phenylbutyric acid product may also be is purified by vacuum distillation. Finally, if desired, the crude 4-phenylbutyric acid is purified by recrystallization using a combination of non-polar solvents. In this process, the mineral acid is preferably hydrochloric acid or sulfuric acid, and the solvent contains a carboxylic acid of less than four carbon atoms in the main chain.

In another embodiment we provide a process of preparing sodium 4-phenylbutyrate including the steps of dissolving 4-phenylbutyric acid in an organic medium, treating the solution with in inorganic alkali such as sodium hydroxide or sodium carbonate, heating the resulting mixture, optionally concentrating the heated mixture by distilling out the solvent, adding a suitable solvent to the mixture to precipitate sodium 4-phenylbutyrate from the mixture, and isolating the product by filtration and drying under vacuum at a selected temperature. The organic medium is selected from one or more organic solvents preferably chosen from the group consisting of alkyl alcohols (such methanol, ethanol, and isopropanol), alkyl esters (such ethyl acetate), and tetrahydrofuran, and compatible mixtures thereof. The preferred temperature at which the solution is first heated is in the range of about 30° C to about 95° C. In the precipitation step, the organic solvent is preferably chosen from the group consisting of dialkyl ethers (such as isopropyl ether and diethyl ether), dialkyl acetates (such as ethyl acetate), dialkyl ketones (such as acetone or ethyl methyl ketone), and other solvents, such as 1,4-dioxan, and compatible mixtures thereof.

Practice of this invention is illustrated by the non-limiting examples provided herein.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 500 mg/mL

Example 1

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was added about 10 mL of water, and the mixture was agitated to dissolve the butyrate and form a solution. To the solution was added about 0.05 g of sodium saccharin, 0.05 g of sodium benzoate, and the solution was mixed well. This solution was compounded with water to yield 25 mL of a liquid oral dosage form.

Example 2

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. About 10 mL of water was added to the flask and the mixture was agitated to dissolve the butyrate. To the solution was added about 0.05 g of raspberry flavor (*e.g.*, raspberry XBF-700194, available from IFF International Flavors & Fragrances, New York, NY), 0.05 g of sodium benzoate, and then mixed well. This mixture was

compounded to 25 mL with water. Any flavoring that is dispersible in water is generally suitable for this invention.

Example 3

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was added about 10 mL of water and agitated to dissolve. To the mixture was added about 0.05 g of sodium benzoate and mixed well. This mixture was compounded to 25 mL with water.

Example 4

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. Added about 10 mL of water and agitated to dissolve. To the mixture added about 0.05 g of raspberry flavoring, 0.05 g of sodium benzoate, 0.05 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 5

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask, to which was added about 10 mL of water and then agitated to dissolve. To the mixture was added about 0.15 g of raspberry flavor, 0.05 g of sodium benzoate, 0.25 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 6

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. To that was added about 10 mL of water and the mixture agitated to dissolve. To the solution was then added about 100 mg of sodium carbonate, 0.15 g of raspberry flavor, 0.05 g of sodium benzoate, 0.25 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 7

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask, about 10 mL of water was added, and the mixture agitated to dissolve. Then were added about: 100 mg of sodium carbonate, 0.15 g of raspberry flavor, 0.05 g of

sodium benzoate, and 0.25 g of sucralose; and the combination mixed well. This mixture was compounded to 25 mL with water.

Example 8

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. About 9 mL of water was added and the mixture agitated with heating to a temperature of about 70° C to dissolve. The solution was then left to cool to room temperature and about 0.05 g of raspberry flavor, 0.05 g of sodium benzoate, and 0.05 g of sodium saccharin were added with good mixing. This mixture was compounded to 25 mL with water.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 640 mg/mL

Example 9

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask and about 9 mL of water was added and the mixture, which was then agitated with heating at a temperature of about 70° C to dissolve the butyrate. The solution was then cooled to 25° C and 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added with good mixing. This solution was compounded to 25 mL with water.

Example 10

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was then added about 9 mL of water. The mixture was agitated to dissolve the butyrate at an elevated temperature of about 70° C . The solution was cooled to 25° C and 0.05 g of sodium benzoate was added and the solution mixed well. This solution was compounded to 25 mL with water.

Example 11

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask. About 90 mL of water was added and the mixture agitated with heating at a temperature 70° C to dissolve. The solution was then cooled to 25° C and 0.5 g of sodium benzoate and 0.5 g of sodium saccharin were added and mixed well. This solution was compounded to 250 mL with water.

Example 12

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask. To the flask was added about 90 mL of water and the mixture agitated with heating at a temperature 70° C to dissolve. The mixture was cooled to 25° C and 0.5 g of sodium benzoate was added and mixed well. This mixture was compounded to 250 mL with water.

Example 13

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask to which was then added about 90 mL of water and agitated with heating at a temperature 70° C to dissolve. The mixture was cooled to 25° C and 0.5 g of sodium benzoate was added and mixed well. This solution was compounded to 250 mL with water. This solution was then kept at 0°C for about 48 hours and was no precipitation or freezing of the solution was found to have occurred. Further cooling of this solution to about –4° C caused precipitation.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 500 mg/mL starting with 4-phenylbutyric acid.

Example 14

About 10.9 g of 4-phenylbutyric acid was transferred to a 25 mL volumetric flask. About 10 mL of water was added and then about 2.9 g of sodium hydroxide was added. This mixture was agitated with heating at a temperature 70° C for about 20 min. until a clear solution resulted. The solution was cooled to 25° C and 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added and mixed well. This solution was compounded to 25 mL with water.

Example 15

About 10.9 g of 4-phenylbutyric acid was transferred to a 25 mL volumetric flask to which was added about 10 mL of water, and about 3.9 g of sodium carbonate was added. This mixture was agitated with heating at a temperature of about 90° C for about 30 min. until a clear solution was obtained. The solution was cooled to 25 ° C and then 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added and

mixed well. This mixture solution compounded to 25 mL with water to provide the liquid oral composition.

Preparation of 4-phenylbutyric acid

Example 16

To a mixture of 2000 mL of acetic acid and 1500 mL of 6N hydrochloric acid was added 500 g of Diester {$PhCH_2CH_2CH(COOEt)_2$). The temperature of the mixture was raised to the range of about 95° to 110° C and refluxed for about 20 hrs. The progress of the reaction was monitored by chromatography, and at completion the acetic acid and water were removed by distillation at atmospheric pressure. The residue was dissolved in water using 10% sodium hydroxide. The aqueous solution was then washed with methylene chloride and the pH was adjusted with concentrated hydrochloric acid to a pH of about 1. The product was extracted with 1700 ml of hexane and the eluate was cooled to -10° C. The resulting precipitated crude 4-phenylbutyric acid was isolated by filtration and dried under vacuum at about 30 °C. Yield 280 g (90%). The crude 4-phenyl butyric acid so isolated was dissolved in 1500mL hexane at a temperature of about 30° to 50° C and then cooled to about –10° C and then stirred for about one hour to precipitate. The pure 4-phenyl butyric acid was then isolated by filtration and dried under vacuum without heating. (Purity >99%.)

Example 17

To a mixture of 2000 mL of acetic acid and 1500 mL of 6N hydrochloric acid added 500 g of Diester {$PhCH_2CH_2CH(COOEt)_2$}. The temperature of the mixture was raised to between about 95° to about 110° C and refluxed for about 20 hrs. The progress of the reaction mixture was monitored by chromatography and at completion the acetic acid and water were removed by distillation at atmospheric pressure. The residue was dissolved in water using 10 % sodium hydroxide. The aqueous solution was washed with methylene chloride and the pH was adjusted with concentrated hydrochloric acid to about one. The product was extracted with 1700 ml of hexane and the solution was cooled to -10° C. The precipitated crude 4-phenylbutyric acid was isolated by filtration and dried under vacuum at about 30 °C . Yield 280 g (90%). The crude 4-phenyl butyric acid was then fractionally distilled under vacuum at about 170 °C. (Purity > 99 %.)

Preparation of Sodium 4-phenylbutyrate

Example 18

About 200 g of 4-phenylbutyric acid was dissolved in 1200 mL of methanol, then 65 g sodium carbonate was added and the mixture heated to about 60 °C for about 45 min. The solution is concentrated to about 1/10th of its original volume and 7000 mL of acetone was added with stirring for about 40min at about 0° C. The precipitated sodium4-phenylbutyrate was filtered and washed with acetone, and dried under vacuum at 30 ° C.

The foregoing description is meant to be illustrative and not limiting. Various changes, modifications, and additions may become apparent to the skilled artisan upon a perusal of this specification, and such are meant to be within the scope and spirit of the invention as defined by the claims.

What is claimed is:

1. A pharmaceutical liquid composition, comprising: a solution of sodium 4-phenylbutyrate in an aqueous medium at a concentration of at least about 300 mg//mL.
2. The composition of claim 1, further comprising a preservative.
3. The composition of claim 1, further comprising a flavoring agent.
4. The composition of claim 1, further comprising a preservative and a flavor.
5. The composition of claim 3, wherein the flavoring agent is a sweetening agent.
6. The composition of claim 4, wherein the flavoring agent is a sweetening agent.
7. The composition of claim 1, further comprising at least two flavoring agents, at least one of said flavoring agents being a sweetening agent, and a preservative.
8. The composition of claim 1, wherein the concentration of sodium 4-phenylbutyrate ranges from about 300 mg/mL to about 700 mg/mL.
9. The composition of claim 8, wherein the concentration of sodium 4-phenylbutyrate is in the range from about 400 mg / mL to about 600 mg/mL.
10. The composition of claim 9, wherein the concentration is about 500 mg/mL.
11. The composition of claim 2, 4, 6, or 7, wherein the preservative is sodium benzoate.
12. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is sodium saccharine.
13. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is sucralose.
14. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is a mixture of sodium saccharine and sucralose
15. The composition of claim 3, 4, 5, or 7, wherein the flavoring agent is a raspberry flavor.
16. The composition of claim 1, further comprising a base.
17. The composition of claim 14, wherein the base is sodium carbonate.
18. The composition of claim 14, wherein the base is sodium hydroxide.
19. The composition of claim 1, further comprising 4-phenylbutyric acid
20. The composition of claim 17, further comprising sodium carbonate.

21. The composition of claim 1, wherein the weight fraction of water is less than the weight fraction of sodium 4-phenylbutyrate.
22. A process for preparing an aqueous solution of 4-phenylbutyrate, comprising the steps of: adding water to sodium 4-phenylbutyrate powder; and dissolving the powder in the water by agitation at temperature ranging from about 25° C to about 80° C to obtain a solution having a concentration of at least about 300 g/mL of 4-phenylbutyrate.
23. The process of claim 20, wherein weight fraction of water in the solution is less than the weight fraction of 4-phenylbutyrate.
24. A process for making of sodium 4-phenylbutyrate, comprising the steps of:
    (A) dissolving 4-phenylbutyric acid in a first organic solvent medium;
    (B) treating the solution of step (A) with a inorganic alkali;
    (C) heating the treated solution of step (B) to a predetermined temperature;
    (D) adding a second solvent to the heated mixture effective to precipitate sodium 4-phenylbutyrate therefrom; and
    (E) isolating the precipitate product by filtration and drying under vacuum at a predetermined temperature.
25. The process of claim 22, further comprising concentrating the solution obtained after step (C) by distilling out the organic solvent medium.
26. The process of claim 22, wherein the inorganic alkali is sodium carbonate.
27. The process of claim 22, wherein the inorganic alkali is sodium hydroxide.
28. The process of claim 22, wherein the first organic solvent comprises two or more organic solvents.
29. The process of claim 22 or 26, wherein the first organic solvent is selected from the group consisting of methanol, ethanol, isopropanol, ethyl acetate, tetrahydrofuran, and compatible mixtures thereof.
30. The process of claim 22, the second solvent is two or more organic solvents.
31. The process of claim 22 or 28, wherein the second solvent is selected from the group consisting of Isopropyl ether, diethylether, ethyl acetate, ethyl methyl ketone, 1,4-dioxan, acetone, and compatible mixtures thereof.
32. The process of claim 22, wherein the predetermined temperature in each of step (C) and (E) is independently selected to be in the range of about 30°C to about 95° C.

33. A process for making 4-phenylbutyric acid, comprising:
    (i) treating an organic ester of the formula Ph-CH_2-CH_2-CH-$(COOR)_2$, wherein each R is independently an alkyl containing up to four carbon atoms, an aryl group, or an aralkyl group wherein the alkyl portion has up to four carbon atoms, with a mineral acid in a water miscible organic solvent at a predetermined temperature; and
    (ii) precipitating 4-phenylbutyric acid using a non-polar solvent..
34. The process of claim 31, further comprising concentrating the solution by evaporation between steps (i) and (ii).
35. The process of claim 31, further comprising step of purifying the crude 4-phenylbutyric acid obtained in step (ii) by vacuum distillation.
36. The process of claim 31, further comprising purifying the crude 4-phenylbutyric acid obtained in step (ii) by recrystallization using one or more solvents.
37. The process of claim 31, wherein each R is independently methyl, ethyl or propyl.
38. The process of claim 34, wherein the water miscible organic solvent contains one or more carboxylic acids having less than 4 carbons in the main chain.
39. The process of claim 36, wherein the carboxylic acids selected from propanoic acid, substituted propanoic acid, acetic acid, substituted acetic acid, and formic acid.
40. The process of claim 36 or 37, wherein the mineral acid is hydrochloric acid.
41. The process of claim 36 or 37, wherein the mineral acid is sulfuric acid.
42. A method of treating a patient suffering from a urea cycle deficiencies, sickle-cell anemia, cancer, or potential cerebral ischemic injury, comprising providing an oral aqueous solution of 4-phenylbutyrate having a concentration of at least about 300 mg/mL and orally administering said solution to a patient in need thereof.
43. The method of claim 40, wherein the solution further comprises a preservative, a flavoring agent, a fragrance, or a mixture thereof.
44. The method of claim 41, wherein the solution further comprises a preservative and a flavoring agent.

45. The method of claim 42, wherein the solution further comprises a fragrance and a sweetener as the flavoring agent.
46. The composition of any of claims 1-10 and 14-19, wherein the solution does not freeze at 0° C.
47. The process of claim 20 or 21, wherein the solution does not freeze at 0° C.

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(54) **Title:** AGONISTS FOR ANTIMICROBIAL PEPTIDE SYSTEMS

(57) **Abstract:** Short chain fatty acids (SCFAs) and glycerol esters of SCFAs not previously used for that purpose are provided for use as a medicament for treating, preventing or counteracting microbial infections in animals, including humans, by stimulating the innate antimicrobial peptide defense system . Preferred compounds include phenyl substituted short chain fatty acids (SCFAs) derivatives and. Also provided are methods and compositions for treating, preventing or counteracting microbial infections, including bacterial, viral, fungal, and parasitic infections, by administration of medicaments comprising a secretagogue-effective amount of the compounds of the invention.

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Agonists for antimicrobial peptide systems

Technical field

The invention relates to compounds which are active as drugs for stimulating the innate antimicrobial peptide system and can be used as antimicrobial drugs.

Background art

Antimicrobial peptides and proteins play an important role in innate host defenses and are believed to be particularly important at mucosal surfaces that form the initial barrier between the host and the external environment. Such peptides are found in large quantities in the colonic epithelium. The peptides can be considered as endogenous antibiotics and are widespread in nature as immediate defense effectors. They are mainly stored in vacuoles of granulocytes ready for activation upon stimulation or secreted directly onto mucosal and other surfaces by epithelial cells.

A human antimicrobial peptide has been identified and is referred to as LL-37, a 37-residue peptide present in neutrophils, epithelial cells and lymphocytes. Both isolated and chemically synthesised LL-37 show antimicrobial activity *in vitro*.

Certain bacteria have evolved mechanisms to overcome the antimicrobial peptide barrier, such as *Shigella* bacteria which down-regulate LL-37 expression in the colon epithelium.

Rabbani *et al. (Short-Chain Fatty Acids Improve Clinical, Pathologic, and Microbiologic Features of Experimental Shigellosis. The Journal of Infectious Diseases 1999;179:390–7)* investigated that naturally occurring short chain fatty acids (SCFAs; acetate, propionate, and butyrate in 60:30:40 ratio) which occur as fermentation products in the gut. The authors used a rabbit model of shigellosis. They reported that the mixture, given by colonic infusion into the rabbits with acute shigellosis, improved clinical, pathologic, and bacteriologic characteristics.

Hase et al. (*Cell Differentiation Is a Key Determinant of Cathelicidin LL-37/Human Cationic Antimicrobial Protein 18 Expression by Human Colon Epithelium. INFECTION AND IMMUNITY, Feb. 2002, vol 70, No 2 p. 953–963*) reported that infection *in vitro* of HCA-7 cells with *Salmonella enterica* serovar Dublin or enteroinvasive *Escherichia coli*

modestly upregulated LL-37/hCAP18 mRNA expression. The authors concluded that differentiated human colon epithelium expresses LL-37/hCAP18 as part of its repertoire of innate defense molecules.

Schauber et al. (*Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways*. Gut 2003; 52:735-741.) investigated the effect of naturally occuring SCFAs on LL-37 expression in vivo and in vitro. These authors report that following exposure to butyrate, isobutyrate and propionate, expression of the LL-37 mRNA increases *in vitro* in colonocytes. The authors are cautious about the possible consequences of increased antimicrobial peptide expression on the commensal intestinal flora, which is critical for protection of the mucosa against enteropathogenic microbes. They note a pathological increase in the activity of endogenous antibiotics would not then be beneficial to the host but might have deleterious consequences.

Raqib *et al.* (*Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic*. Proc. Natl. Acad. Sci. 2006; 103: 9178-9183.) reported that butyrate treatment of rabbits resulted in reduced clinical illness and bacterial load in the stool and significant upregulation of CAP-18 (the rabbit homologue of LL-37) in the surface epithelium.

Other molecules have also been investigated for their possible utility in stimulating natural defensins.

WO2000-09137 (Magainin Pharmaceuticals) describes newly isolated aminosterol compounds and pharmaceutical compositions based on the aminosterol compounds are described. Methods for the treatment of various disorders, for example, a microbial infection, are also described

US2002-0076393 (Fehlbaum *et al.*) describe the use of isoleucine or active isomers or analogs thereof for stimulating production of defensin. It should be noted that the claims refer, *inter alia*, to one such analog being butyrate or an active derivative thereof. However where butyrate was tested and it appeared to be less active than isoleucine at similar concentrations (see Figure 7 therein).

US2003-0109582 (Zasloff) describe the use of isoleucine compounds for stimulating Paneth cells to release natural antimicrobial agents including peptides, to reduce or eliminate pathogenic organisms in the GI tract of mammalian bodies, including humans, utilizing an active isoleucine compound as a secretagogue. "Isoleucine compounds" are defined as including 'isoleucine butyrate' though this compound is not described or tested.

US7311925 (Zasloff) describes methods of blocking microbial adherence to a eukaryotic cell surface in a mammal by applying a pharmacologically acceptable composition containing at least one compound selected from the group consisting of isoleucine, an active isomer thereof, and an active analog thereof, to said surface in a microbial blocking quantity. Active analogs of isoleucine are defined as including 'isoleucine butyrate' though this compound is not described or tested.

US20080038374 (Stahle) describes use of a vitamin D compound, which is able to specifically and directly up-regulate hCAP18, for the manufacturing of a medicament with antimicrobial effect for treatment of conditions deficient in LL-37, such as chronical ulcers, and atopic dermatitis.

WO/2008/073174 (GALLO) describes methods and compositions for modulating gene expression and cathelicidin the innate immune response by $1,25(OH)_2$ vitamin D3 (1,25D3). That compound is tested alongside non-specific histone deacetylase inhibitors (HDACi) including butyrate or trichostatin A.

Hata *et al.* (2008) "*Administration of oral vitamin D induces cathelicidin production in atopic individuals*" J ALLERGY CLIN IMMUNOL, VOLUME 122, NUMBER 4, described a study in which 14 normal controls and 14 atopic subjects with moderate to severe atopic dermatitis were treated with oral vitamin D3 to see if this could overcome the relative deficiency in induction of cathelicidin in the atopic patients. After supplementation with 4000 IU/d oral vitamin D for 21 days, AD lesional skin showed a statistically significant increase in cathelicidin expression.

Despite the above disclosures, it will be appreciated that the provision of compounds or combinations of compounds for use in enhancing the innate immune response, for example in the gut, would provide a contribution to the art.

Summary of the invention

As can be seen from the discussion above, the publications in the art had been cautious about the possible deleterious consequences of SCFA compounds which stimulate the effect of endogenous antibiotics in the human gut, because of their potential effect on commensal intestinal flora. Additionally, it was known that butyrate, for example, had practical drawbacks, in particular the unpleasant odour and taste, that made it unsuitable for pharmaceutical use. These reasons may account for the fact that the effect of SCFAs had not been investigated in the art in humans but greater interest has apparently been given to the use of vitamin D in the skin.

The present inventors have found that a number of pharmaceutically acceptable SCFA-derivatives and prodrugs are active as drugs to stimulate the innate antimicrobial peptide system in human cell lines and can be used as preventive and curative antimicrobial drugs in animal models of disease. These pharmaceutically acceptable SCFA-derivatives may be more acceptable (in terms of odour and\or taste) than butyrate. These findings have profound implications for the use of these compounds on replacing or supplementing existing antibiotics or other antimicrobial strategies in treating human disease.

An abstract has previously been made available stating that an unidentified drug stimulated cathelcidin antimicrobial peptide (CAMP) and human beta-defensin 1 (hBD-1) gene expression in the bronchial epithelial cell line VA10 ("Induction of Antimicrobial Peptide Gene Expression by a approved drug in a Bronchial Epithelial Cell Line"; Jónas Steinmann and Guðmundur Hrafn Guðmundsson, Institute of Biology, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland).

After the presently claimed priority date, a poster was presented showing for the first time that 4-phenylbutyrate (PBA) stimulates cathelicidin antimicrobial peptide gene expression in a bronchial epithelial cell line ("Induction of Antimicrobial Peptide Gene Expression in a Bronchial Epithelial Cell Line"; Jonas Steinmann and Guðmundur Hrafn Guðmundsson Institute of Biology, University of Iceland, 101 Reykjavik, Iceland; 15th March 2008).

Sodium phenylbutyrate is a known medicament. For example it has been marketed by Ucyclyd Pharma (Hunt Valley, USA) under the trade name Buphenyl and by Swedish Orphan International (Sweden) as Ammonaps. It has been used to treat urea cycle

disorders (Batshaw *et al.* (2001) *J. Pediatr.* 138 (1 Suppl): S46–54; discussion S54–5). Scandinavian Formulas, Inc. Sellersville, PA supplies sodium phenylbutyrate worldwide for clinical trials. Sodium phenylbutyrate is also under investigation for the treatment of some sickle-cell disorders (Blood Products Plasma Expanders and Haemostatics) and for use as a potential differentiation-inducing agent in malignant glioma and acute myeloid leukaemia. It has also been investigated in respect of cystic fibrosis pathology due to its capacity to traffic DeltaF508-cystic fibrosis transmembrane conductance regulator (CFTR) to the cell membrane and restore CFTR chloride function at the plasma membrane of CF lung cells in vitro and in vivo (Roque *et al.* J Pharmacol Exp Ther. 2008 Sep;326(3):949-56. Epub 2008 Jun 23). It is believed in the literature that phenylbutyrate is a prodrug which is metabolized in the body by beta-oxidation to phenylacetate.

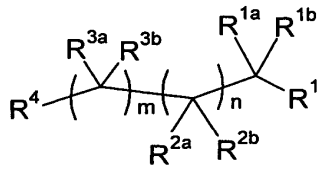
Notwithstanding the above, prior to the present invention, PBA was not known or suggested for the uses claimed herein.

Detailed disclosure of the invention

Thus in a first aspect, the present invention provides compounds as defined by formula I for use as a medicament for treating, preventing or counteracting microbial infections in humans and animals by stimulating the innate antimicrobial peptide defense system,

Compounds of the invention

In a first aspect, the present invention provides compounds as defined by formula Ia for use as a medicament for treating, preventing or counteracting microbial infections in humans and animals by stimulating the innate antimicrobial peptide defense system,



(Ia)

wherein

R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof;

m and *n* are each independently 0 or 1;

R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a} and R^{3b} each independently represent hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group; and/or
R^{2a}, together with an adjacent R^{3a} or R^{1a}, may represent a carbon-carbon π bond; and/or
R^{2b}, together with an adjacent R^{3b} or R^{1b}, may represent a carbon-carbon π bond;

R^4 may be hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group;

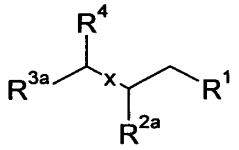
R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group;

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group; and

R^7 is a side chain of a naturally occurring amino acid or is selected from $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, or $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms;

and wherein, if R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is selected from halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group.

In some embodiments the compound may be a compound of formula I:



(I)

wherein, preferably, R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

x represents a single, double or triple bond,

or x-$R^{3a}R^4$ together represent hydrogen in which case R^1 is preferably $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms.

Compounds of formula I are compounds of formula Ia in which R^{1a} and R^{1b} are both hydrogen, *m* and *n* are both 1, and R^{2b} and R^{3b} are *either* both hydrogen *or* together form a π bond in position 'x'. If R^{2a} and R^{3a} also together form a π bond, then position 'x' represents a double bond.

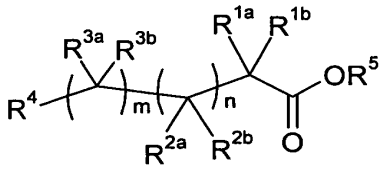
Compounds of formula Ia in which R^{1a}, R^{1b} and R^{2b} are all hydrogen, *m* is 0, *n* is 1, and R^4 is hydrogen can also be represented as compounds of formula I where x-$R^{3a}R^4$ together represent hydrogen.

In compounds of formula I, 'x' is preferably a single bond.

Preferences for R^1

In certain preferred embodiments, the compound of the invention is a carboxylic acid, in these cases R^1 represents a carboxyl group, or a pharmaceutically acceptable salt thereof. If R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen. In other preferred embodiments, R^1 is a carboxylic acid derivative, such as an ester or an amide.

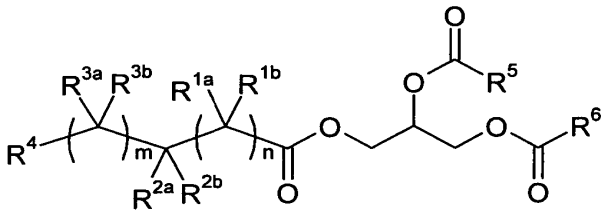
In some such embodiments, as represented by formula IIa, R^1 is an ester group of formula $COOR^5$ where R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, and preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl. Particularly preferred R^5 groups are methyl and ethyl.



(IIa)

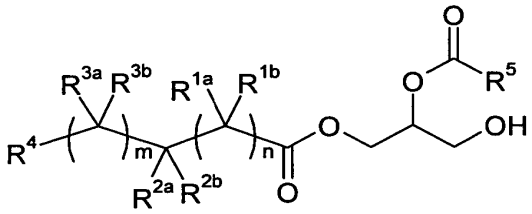
In some preferred embodiments R^1 is an ester selected from a triglyceride ester moiety or diglyceride ester moiety.

If R^1 is a triglyceride moiety the compounds of the invention are of the following general formula (IIb):



(IIb)

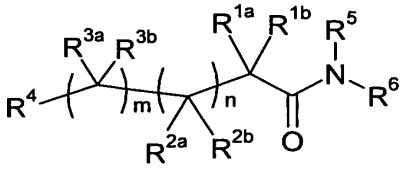
If R^1 is a diglyceride moiety, the compounds of the invention are of the following general formula (IIc):



(IIc)

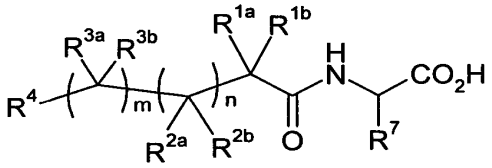
Embodiments of particular interest include glyceryl tributyrate or glyceryl tripropionate. Other preferred embodiments make use of corresponding glycerol esters of one or more phenyl substituted fatty acids or other short chain fatty acids such as the above mentioned. Such glyceryl triesters include for example but not limited to glyceryl tributyrate wherein one or more of the butyrate acyl chains are substituted with phenyl, e.g. 1-butanoyloxy-3-(4'-phenylbutanoyloxy)propan-2-yl butanoate, 1,3-(4',4''-diphenyl)-di(butanoyloxy)propan-2-yl butanoate, and 1,3-di(butanoyloxy)propan-2-yl-4-phenylbutanoate.

Further embodiments which are carboxylic derivatives embodiments include amides of formula (IId), wherein R^1 is a group of formula $CONR^5R^6$, wherein R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl, and R^6 is selected from hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl.



(IId)

In certain embodiments R^1 is an amino acid group, in which case the compounds of the invention may be represented as compounds of the following general formula (IIe):



(IIe)

or a salt thereof, in which R^7 is an amino acid side chain. In some embodiments R^7 is the side chain of a naturally occurring amino acid.

For example, R^7 may be a side chain of leucine ($CH_2CH_2CH_2CH_3$), isoleucine ($CH(CH_3)CH_2CH_3$), methionine ($-CH_2CH_2SCH_3$), lysine ($-CH_2CH_2CH_2CH_2NH_2$), or arginine ($-CH_2CH_2CH_2NHC(=NH)NH_2$). In some embodiments, particularly if R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are all hydrogen and *m* and *n* are 1, R^7 is preferably not an isoleucine side chain ($CH(CH_3)CH_2CH_3$).

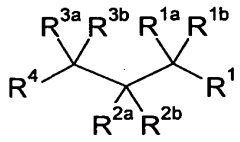
Alternatively, R^7 may be a derivative or analogue of a naturally occurring amino acid side chain, such as a lysine side chain derivative ($-CH_2CH_2CH_2CH_2NHR^8$), an arginine side chain derivative ($-CH_2CH_2CH_2NHC(=NH)NHR^8$), or a group such as $-CH_2CH_2CH_2NHR^8$, wherein R^8 represents hydrogen, a linear or branched substituted or unsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group.

In certain embodiments found to be useful, the compounds of the invention are relatively small SCFA derivatives. For example, compounds of formula I wherein R^{2a} and R^4 represent hydrogen. In these embodiments R^{3a} is preferably hydrogen, hydroxyl, or a substituted or nonsubstituted aryl group including phenyl, or benzyl, with the above

limitation applying to R^{3a} in the case where R^1 is carboxyl or a salt thereof. Substituted aryl can be hydroxyl or amino-substituted phenyl, or benzyl.

Preferred chain lengths

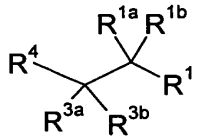
In some preferred compounds of the invention, *m* and *n* are each 1. These compounds may be described as butyric acid/butyrate derivatives and are of general formula (IIIa):



(IIIa)

where R^1, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are as previously defined.

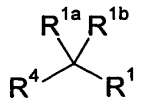
In other preferred compounds, *m* is 1 and *n* is 0. These compounds may be described as propionic acid/propionate derivatives and are of general formula (IIIb):



(IIIb)

where R^1, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are as previously defined. It can be seen that if *m* were 0 and *n* were 1, this would also result in propionic acid derivatives.

In some embodiments, both *m* and *n* may be 0. This results in compounds which may be described as acetic acid/acetate derivatives, of general formula (IIIc):

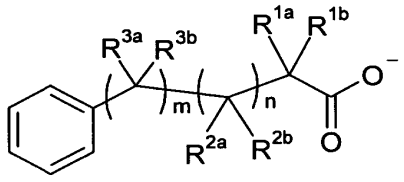


(IIIc)

Preferred substituents

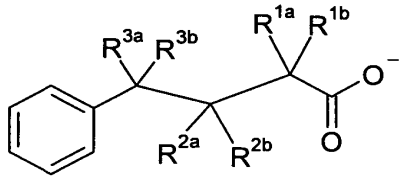
Preferred embodiments of the invention include compounds which are substituted butyric, propionic or acetic acid derivatives of general formulae (IIIa) to (IIIc), wherein R^1 is carboxylate or a derivative thereof as defined above and wherein one or more of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen, preferably selected from an alkyl group or an aryl group. It is preferred that one or more, preferably one, of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group, most preferably a phenyl or substituted phenyl group. When one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group, it is preferred that the others are selected from hydrogen or an alkyl group, the alkyl group being preferably methyl.

Most preferably, R^4 is an aryl group, preferably phenyl or substituted phenyl. Certain preferred compounds according to these embodiments are of general formula (IVa):



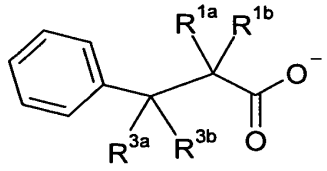
(IVa)

Preferred butyric acid derivatives are therefore of general formula (IVb):



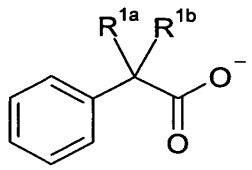
(IVb)

preferred propionic acid derivatives are of general formula (IVc):



(IVc)

and preferred acetate derivatives are of general formula (IVd):



(IVd)

In formulae (IVa) to (IVd), the phenyl ring may optionally be substituted with one or more substituents, as further defined below. Preferred substituents are alkyl, halide, hydroxyl and amino.

The carboxylate group may optionally be derivatised as an ester or amide, as set out above. In these embodiments, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} are preferably hydrogen or an alkyl group with 1 to 10 carbon atoms, the alkyl group being preferably methyl or ethyl.

In alternative embodiments, R^4 may be hydrogen, and one or more, preferably one, of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} may be an aryl group such as phenyl or substituted phenyl.

Substituents α to the carboxylate

R^{1a} and R^{1b} are preferably selected from hydrogen and an alkyl group having from 1 to 10 carbon atoms, the alkyl group being preferably methyl or ethyl. In some embodiments, R^{1a} and R^{1b} may both be alkyl, but it is preferred that at least one of R^{1a} and R^{1b} is hydrogen.

In particular, the following compounds are useful in accordance with the invention: 4-phenylbutyric acid, 3-phenylbutyric acid, 2-phenylbutyric acid, 3-phenylpropionic acid, 2-phenylpropionic acid, 2-methyl-3-phenylpropionic acid *[ST7]*, 2-methyl-4-phenylbutyric acid, or a pharmaceutically acceptable salt of any of said compounds, methyl 4-phenylbutyrate, ethyl 4-phenylbutyrate, methyl 3-phenylbutyrate, ethyl 3-phenylbutyrate, methyl 2-phenylbutyrate, ethyl 2-phenylbutyrate, methyl 3-phenylpropionate, ethyl 3-phenylpropionate, methyl 2-phenylpropionate, ethyl 2-phenylpropionate, methyl 2-methyl-3-phenylpropionate, ethyl 2-methyl-3-phenylpropionate, methyl 2-methyl-4-phenylbutyrate, and ethyl 2-methyl-4-phenylbutyrate.

Metabolites of these compounds may also be useful in the invention, in particular phenyl acetate.

Substituents β to the carboxylate (where present)

In embodiments, one or both of R^{2a} and R^{2b} may optionally be hydroxyl. This may be preferred where it is desired that the compound of the invention have increased resistance to metabolism such as beta oxidation, and hence in principle a longer half-life.

Definitions and further preferences

Alkyl:
As used herein the term "alkyl", unless otherwise specified, refers to a C_{1-10} alkyl group, that is to say a monovalent moiety obtained by removing a hydrogen atom from a hydrocarbon compound having from 1 to 10 carbon atoms, which may be aliphatic or alicyclic, or a combination thereof, which may be linear or branched, and which may be saturated, partially unsaturated, or fully unsaturated. In certain instances C_{1-4}, C_{1-5}, C_{1-6} or C_{1-7} alkyl groups may be preferred.

Examples of saturated linear C_{1-10} alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, n-pentyl (amyl) and n-hexyl.

Examples of saturated branched C_{1-10} alkyl groups include, but are not limited to, iso-propyl, iso-butyl, sec-butyl, tert-butyl, and neo-pentyl.

Examples of saturated alicyclic C_{1-10} alkyl groups (which may also be referred to as "C_{3-10} cycloalkyl" groups) include, but are not limited to, groups such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl, as well as substituted groups (e.g., groups which comprise such groups), such as methylcyclopropyl, dimethylcyclopropyl, methylcyclobutyl, dimethylcyclobutyl, methylcyclopentyl, dimethylcyclopentyl, methylcyclohexyl, dimethylcyclohexyl, cyclopropylmethyl and cyclohexylmethyl.

Unsaturated alkyl groups contain one or more double or triple bonds i.e. one or more carbon-carbon π bonds. Examples of unsaturated C_{1-10} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{2-10}alkenyl" groups) include, but are not limited to, ethenyl (vinyl, -CH=CH_2), 2-propenyl (allyl, -CH-CH=CH_2), isopropenyl (-C(CH_3)=CH_2), butenyl, pentenyl, and hexenyl.

Examples of unsaturated C_{1-10} alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C_{2-10} alkynyl" groups) include, but are not limited to, ethynyl (ethinyl) and 2-propynyl (propargyl).

Examples of unsaturated alicyclic (carbocyclic) C_{1-10} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{3-10}cycloalkenyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl, as well as substituted groups (e.g., groups which comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

Aryl:

As used herein the term "aryl", unless otherwise specified, refers to a C_{5-20} aryl group, that is to say a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups", in which case the group may conveniently be referred to as a "C_{5-20} carboaryl" group.

Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e. C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), naphthacene (C_{18}), and pyrene (C_{16}).

Examples of aryl groups which comprise fused rings, one of which is not an aromatic ring, include, but are not limited to, groups derived from indene and fluorene.

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulphur, as in "heteroaryl groups". In this case, the group may conveniently be referred to as a "C_{5-20} heteroaryl" group, wherein "C_{5-20}" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C_{5-20} heteroaryl groups include, but are not limited to, C_5 heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole),

pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, and oxatriazole; and C_6 heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine), triazine, tetrazole, and oxadiazole (furazan).

Examples of C_{5-20} heteroaryl groups which comprise fused rings, include, but are not limited to, C_9 heterocyclic groups derived from benzofuran, isobenzofuran, indole, isoindole, purine (e.g., adenine, guanine), benzothiophene, benzimidazole; C_{10} heterocyclic groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine, quinoxaline; C_{13} heterocyclic groups derived from carbazole, dibenzothiophene, dibenzofuran; C_{14} heterocyclic groups derived from acridine, xanthene, phenoxathiin, phenazine, phenoxazine, phenothiazine.

Optional Substitution:

The above alkyl and aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkoxy group, discussed below), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7} alkyl group.

C_{1-7} alkoxy: -OR, wherein R is a C_{1-7} alkyl group. Examples of C_{1-7} alkoxy groups include, but are not limited to, $-OCH_3$ (methoxy), $-OCH_2CH_3$ (ethoxy) and $-OC(CH_3)_3$ (tert-butoxy).

Oxo (keto, -one): =O; carbonyl (>C=O). Examples of cyclic compounds and/or groups having, as a substituent, an oxo group (=O) include, but are not limited to, carbocyclics such as cyclopentanone and cyclohexanone; heterocyclics, such as pyrone, pyrrolidone, pyrazolone, pyrazolinone, piperidone, piperidinedione, piperazinedione, and imidazolidone; cyclic anhydrides, including but not limited to maleic anhydride and succinic anhydride; cyclic carbonates, such as propylene carbonate; imides, including but

not limited to, succinimide and maleimide; lactones (cyclic esters, -O-C(=O)- in a ring), including, but not limited to, β-propiolactone, γ-butyrolactone, δ-valerolactone, and ε-caprolactone; and lactams (cyclic amides, -NH-C(=O)- in a ring), including, but not limited to, β-propiolactam, γ-butyrolactam (2-pyrrolidone), δ-valerolactam, and ε-caprolactam.

Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, C_{1-7} alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, =NH, =NMe, =NEt, and =NPh.

Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C_{1-7}alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (butyryl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -COOH.

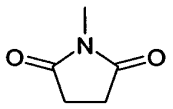
Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7}alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and -C(=O)OPh.

Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7}alkyl group. Examples of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, -OC(=O)Ph, and $-OC(=O)CH_2Ph$.

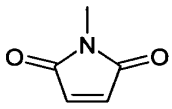
Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^{N1} and

R^{N2}, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

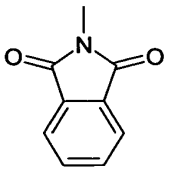
Acylamido (acylamino): $-NR^{A1}C(=O)R^{A2}$, wherein R^{A1} is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group, and R^{A2} is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and -NHC(=O)Ph. R^{A1} and R^{A2} may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl and phthalimidyl:



succinimidyl



maleimidyl



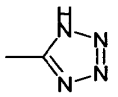
phthalimidyl

Acylureido: $-N(R^{U1})C(O)NR^{U2}C(O)R^{A3}$ wherein R^{U1} and R^{U2} are independently ureido substituents, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. R^{A3} is an acyl group as defined for acyl groups. Examples of acylureido groups include, but are not limited to, -NHCONHC(O)H, -NHCONMeC(O)H, -NHCONEtC(O)H, -NHCONMeC(O)Me, -NHCONEtC(O)Et, -NMeCONHC(O)Et, -NMeCONHC(O)Me, -NMeCONHC(O)Et, -NMeCONMeC(O)Me, –NMeCONEtC(O)Et, and -NMeCONHC(O)Ph.

Carbamate: $-NR^{N1}$-C(O)-OR^{O2} wherein R^{N1} is an amino substituent as defined for amino groups and R^{O2} is an ester group as defined for ester groups. Examples of carbamate groups include, but are not limited to, -NH-C(O)-O-Me, -NMe-C(O)-O-Me, -NH-C(O)-O-Et, -NMe-C(O)-O-t-butyl, and -NH-C(O)-O-Ph.

Thioamido (thiocarbamyl): $-C(=S)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)N(CH_3)_2$, and $-C(=S)NHCH_2CH_3$.

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,



Amino: $-NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7}alkyl group, or, in the case of a "cyclic" amino group, R^{N1} and R^{N2}, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

Imino: =NR, wherein R is an imino substituent, for example, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group.

Amidine: $-C(=NR)NR_2$, wherein each R is an amidine substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group. An example of an amidine group is $-C(=NH)NH_2$.

Carbazoyl (hydrazinocarbonyl): $-C(O)-NN-R^{N1}$ wherein R^{N1} is an amino substituent as defined for amino groups. Examples of azino groups include, but are not limited to, -C(O)-NN-H, -C(O)-NN-Me, -C(O)-NN-Et, -C(O)-NN-Ph, and $-C(O)-NN-CH_2-Ph$.

Nitro: $-NO_2$.

Nitroso: -NO.

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group (also referred to herein as C_{1-7} alkyl disulfide). Examples of C_{1-7} alkyl disulfide groups include, but are not limited to, $-SSCH_3$ and $-SSCH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$ (triflyl), $-S(=O)_2CH_2CH_3$, $-S(=O)_2C_4F_9$ (nonaflyl), $-S(=O)_2CH_2CF_3$ (tresyl), $-S(=O)_2Ph$ (phenylsulfonyl), 4-methylphenylsulfonyl (tosyl), 4-bromophenylsulfonyl (brosyl), and 4-nitrophenyl (nosyl).

Sulfine (sulfinyl, sulfoxide): -S(=O)R, wherein R is a sulfine substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfine groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ and $-OS(=O)_2CH_2CH_3$.

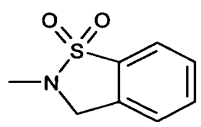
Sulfinyloxy: -OS(=O)R, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

Sulfamino: $-NR^{N1}S(=O)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

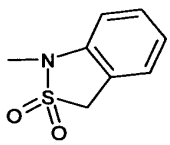
Sulfinamino: $-NR^{N1}S(=O)R$, wherein R^{N1} is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinamino groups include, but are not limited to, $-NHS(=O)CH_3$ and $-N(CH_3)S(=O)C_6H_5$.

Sulfamyl: $-S(=O)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, $-S(=O)NH_2$, $-S(=O)NH(CH_3)$, $-S(=O)N(CH_3)_2$, $-S(=O)NH(CH_2CH_3)$, $-S(=O)N(CH_2CH_3)_2$, and -S(=O)NHPh.

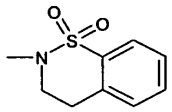
Sulfonamino: $-NR^{N1}S(=O)_2R$, wherein R^{N1} is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonamino groups include, but are not limited to, $-NHS(=O)_2CH_3$ and $-N(CH_3)S(=O)_2C_6H_5$. A special class of sulfonamino groups are those derived from sultams – in these groups one of R^1 and R is a C_{5-20} aryl group, preferably phenyl, whilst the other of R^1 and R is a bidentate group which links to the C_{5-20} aryl group, such as a bidentate group derived from a C_{1-7} alkyl group. Examples of such groups include, but are not limited to:



2,3-dihydro-tenzo[d]isothiazole-1,1-dioxide-2-yl



1,3-dihydro-benzo[c]isothiazole-2,2-dioxide-1-yl



3,4-dihydro-2H-benzo[e][1,2]thiazine-1,1-dioxide-2-yl

Phosphoramidite: $-OP(OR^{P1})-NR^{P2}_2$, where R^{P1} and R^{P2} are phosphoramidite substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidite groups include, but are not limited to, $-OP(OCH_2CH_3)-N(CH_3)_2$, $-OP(OCH_2CH_3)-N(i\text{-}Pr)_2$, and $-OP(OCH_2CH_2CN)-N(i\text{-}Pr)_2$.

Phosphoramidate: $-OP(=O)(OR^{P1})-NR^{P2}_2$, where R^{P1} and R^{P2} are phosphoramidate substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidate groups include, but are not limited to, $-OP(=O)(OCH_2CH_3)-N(CH_3)_2$, $-OP(=O)(OCH_2CH_3)-N(i\text{-}Pr)_2$, and $-OP(=O)(OCH_2CH_2CN)-N(i\text{-}Pr)_2$.

In many cases, substituents may themselves be substituted. For example, a C_{1-7} alkoxy group may be substituted with, for example, a C_{1-7} alkyl (also referred to as a C_{1-7} alkyl-C_{1-7}alkoxy group), for example, cyclohexylmethoxy, a C_{3-20} heterocyclyl group (also referred to as a C_{5-20} aryl-C_{1-7} alkoxy group), for example phthalimidoethoxy, or a C_{5-20} aryl group (also referred to as a C_{5-20}aryl-C_{1-7}alkoxy group), for example, benzyloxy.

Preferred substituents for an aryl or alkyl group may include C_{1-10} alkyl groups, C_{5-20} aryl groups, hydroxyl, C_{1-7}alkoxy groups, nitro, amino, substituted amino ($-NR^{N1}R^{N2}$ as defined above) and halides.

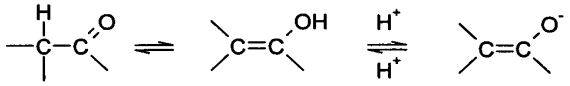
Isomers, Salts, Solvates, and Protected Forms

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and

halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.



keto enol enolate

Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g., fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al.*, *J. Pharm. Sci.*, 66, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g., $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulphuric, sulphurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, glycolic, stearic, palmitic, lactic, malic, pamoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicyclic, sulfanilic, 2-acetyoxybenzoic, fumaric, phenylsulfonic, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, pantothenic, isethionic, valeric, lactobionic, and gluconic. Examples of suitable polymeric anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form", as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts, Wiley, 1999).

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group (>C=O) is converted to a diether (>C($OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide (-NHCO-CH_3); a benzyloxy amide (-NHCO-$OCH_2C_6H_5$, -NH-Cbz); as a t-butoxy amide (-NHCO-$OC(CH_3)_3$, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-$OC(CH_3)_2C_6H_4C_6H_5$, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases, as an N-oxide (>NO•).

For example, a carboxylic acid group may be protected as an ester for example, as: an C_{1-7} alkyl ester (e.g. a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g., a C_{1-7} trihaloalkyl ester); a triC_{1-7} alkylsilyl-C_{1-7} alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether ($-S-CH_2NHC(=O)CH_3$).

Prodrugs

It is contemplated that some of the active compounds of the invention act in the form of prodrugs, that means that they are metabolised in the body to the active form. Among these compounds are esters such as glyceryl tributyrate, glyceryl tripropionate, glyceryl tri(4-phenylbutyrate) and methyl 4-phenylbutyrate.

Further aspects and embodiments

In the following aspects or embodiments of the invention the compound of the invention is any as defined above e.g. as in formula Ia or formula I, or IIIa.

Preferably the compound is a butyric acid/butyrate derivative such as an acid salt, ester or amide such as is defined by any of formula IIa, IIb, IIc, IId, IIe.

Preferably it comprises at least one aryl substituent, which is preferably at R^4, such as is defined by any of formula IVb.

In particular aspects of the invention there are provided methods for treating, preventing or counteracting a microbial infection in a patient in need of the same, by administering to the patient an effective amount of a compound of the invention as described herein.

The effective amount is sufficient to demonstrate antimicrobial activity *in vivo* e.g. by stimulating (e.g. derepressing or inhibiting down-regulation of) synthesis of the cathelicidin LL-37. Stimulation may be towards, equal to, or above basal levels (i.e. normal levels in the absence of the infection).

By the term "antimicrobial activity" as used herein, is meant the ability to inhibit the growth of or actually kill a population of microbes which can be bacteria, viruses, protozoa or fungal microbes. Thus "antimicrobial activity" should be construed to mean both microbistatic as well as microbicidal activities. Antimicrobial activity should also be construed to include a compound which is capable of inhibiting infections, i.e. disease-causing capacity of microbes.

The compounds of the present invention exhibit an antimicrobial effect by stimulating the innate antimicrobial peptide defense system.

Generally the use of the present invention will be such as to lead to secretion of the relevant peptide same onto an epithelial surface (e.g. in the gastrointestinal tract). This in turn will lead to increased antimicrobial activity at the surface (and hence improvement of its barrier function) and treatment of the microbial infection and disease caused by it.

The microbial targets and diseases targeted by the present invention may be any believed to benefit therefrom, but a preferred target is infectious colitis e.g. as caused by Clostridium difficile colitis.

The compounds of the invention are particularly useful against infections of bacterial strains that are tolerant against conventional antibiotics. Nevertheless use of the compounds described herein in conjunction with conventional antibiotics may be preferred and forms one part of the present invention.

Other combination treatments of the present invention include the use of compounds described herein with other other compounds believed to have antimicrobial effect. These include: aminosterol type compounds, for example which include spermidine, spermine or other polyamines (see WO2000-09137); isoleucine or active isomers or analogs thereof (see US2002-0076393 or US2003-0109582 or US7311925); and vitamin D type compounds (see US20080038374 or WO/2008/073174). The disclosure of all these references, in respect of these compounds, their definition, and their provision, is hereby specifically incorporated herein by cross-reference.

Preferred dosages and dosage forms are described in more detail below. A preferred daily dosage may be between 250 µg to about 25 g, preferably up to around 5g, more preferably less than 3 g per day, which may be split into doses given e.g. 1, 2 or 3 times daily.

Said compound is preferably administered in an oral dosage form such as but not limited to a tablet, a capsule, a solution, a suspension, a powder, a paste, an elixir, and a syrup. Other administration forms are also useful, these include but not are limited to topical administration forms, which are in particular useful against infections of the skin, these

include for example creams, oils, lotions, and ointments. Yet further dosage forms include dosage forms for delivery to the respiratory system including the lungs, such as aerosols and nasal spray devices.

Aspects of the invention include a method for treating, preventing or counteracting microbial infections, including bacterial, viral, fungal and parasitic infections (also including infections by bacterial strains resistant to currently used antibiotics), by administering a medicament comprising a secretagogue-effective amount of at least one compound of the invention as defined above.

In yet a further aspect, the invention provides a pharmaceutical composition for use in the methods described herein e.g. for treating, preventing or counteracting a microbial infection, including the above mentioned types, comprising an active ingredient being at least one compound of the invention, and typically at least one pharmaceutically acceptable excipient.

In yet a further aspect, the invention provides use of compounds of the invention in the preparation of a medicament for use in the methods described herein.

Some of these aspects and embodiments will now be discussed in more detail:

Secretion of host defense peptides

The gastrointestinal tract (GI tract) of mammals is covered by a continuous sheet of epithelial cells that is folded into villus projections and crypts. Within the base of the crypts, where the stem cells of the GI tract can be found, there are specialized, granular cells called Paneth cells. Both enterocytes and Paneth cells produce antimicrobial peptides. The enterocytes synthesize and secrete antimicrobial peptides into the gut lumen both constitutively and upon induction. The Paneth cells at the base of the intestinal crypts, secrete alpha-defensins into the cryptal well, resulting in concentrations estimated at mg/mL levels, which eventually flush into the gut lumen.

Both systems contribute to bowel health. In children and adults suffering from diarrhea caused by Shigella, synthesis of the cathelicidin LL-37 and the colonic enterocyte beta-defensin HBD-1 is markedly depressed; expression recovers in time during resolution of the illness. Similarly, mice which lack the proteolytic enzyme required for processing

cryptdins (the murine Paneth cell alpha-defensins) lack functional cryptdins and exhibit increased susceptibility to orally administered Salmonella.

Other epithelial surfaces of the mammalian body also have such host defense secretion systems, including but not limited to the cornea, the lung, the kidney and the skin.

The use of the compositions and methods of the present invention result in the stimulation of epithelial cells and Paneth cells of the gastrointestinal tract and other epithelial surfaces of man and in other animals to secrete large quantities of naturally occurring broad-spectrum antimicrobial agents, including antimicrobial peptides such as defensins, cryptdins, LL-37, HBD1, and HBD2, and antimicrobial proteins such as lysozyme, transferrin, lactoferrin, phospholipases, and SLPI (secretory leukocyte protease inhibitor). The substances stored by the Paneth cells exhibit activity against a wide range of infectious agents including bacteria, protozoa, viruses, and fungi.

The epithelial cells targeted by the present invention may be any of these. Preferably however the invention is utilise for the treatment of microbial infections of the GI tract.

Microbial infections and diseases

As mentioned, an important aspect of the invention provides methods for treating, preventing or counteracting microbial infections by administering a medicament comprising a secretagogue-effective amount of at least one compound of the invention.

In useful embodiments, infections and other conditions that benefit from treatment according to the invention are in particular those relating to organs having epithelial surfaces with host defense peptide secretion systems such as the above mentioned.

Such infections, conditions and diseases include but are not limited to traveller's diarrhoea, endemic diarrhoea, dysentery, viral gastroenteritis, parasitic enteritis, Crohn's disease, ulcerative colitis, irritable bowel syndrome, precancerous states of the gastrointestinal tract, cancer of the gastrointestinal tract, diverticulitis, post-antibiotic diarrhoea, Clostridium difficile colitis, lactose intolerance, flatulence, gastritis, esophagitis, heartburn, gastric ulcer, ulcers associated with Helicobacter pylori, duodenal ulcer, short bowel syndrome, dumping syndrome, gluten enteropathy, or food intolerance.

Also included in the methods of the inventions are infections of the skin, including but not limited to boils, carbuncles, furuncles, cellulitis, abscesses, impetigo, and erysipelas; infections of the eye including but not limited to conjunctivitis, stye, blepharitis, cellulitis, keratitis, corneal ulcer, trachoma, uveitis, canaliculitis and dacryocystitis, infections to the respiratory system and infections in the kidneys. Also included are infections caused by bacterial strains resistant to classical antibiotic treatment, including infections by multidrug resistant strains.

A preferred target for the present invention is infectious colitis. As is well known in the art, microbial species causing this include Yersenia enterocolitica, Salmonella, Shigella, Campylobacter, Clostridium and E. Coli. Some bacteria, such as Clostridium difficile, may elaborate a toxic substance that leads to the development of pseudomembranous colitis.

The compounds of the invention are particularly useful against infections of bacterial strains that are tolerant against conventional antibiotics, and it follows from the secretagogue action of the compounds in the context herein, that it is not foreseen that bacterial strains can develop resistance against treatment in accordance with the invention.

As illustrated in the accompanying Examples, selected representative compounds have been tested and found to exhibit the desired activity.

Combination treatments

As noted above, use of the use of the compounds described herein in conjunction with conventional antibiotics may be preferred and forms one part of the present invention. Example antibiotics include Penicillins, Penicillin G, Phenoxymethyl– penicillin, Flucloxacillin, Amoxycillin, Metronidazole, Cefuroxime, Augmentin, Pivmecillinam, Acetomycin, Ciprofloxacin and Erythromycin. Where these specific antibiotics are named, it will be appreciated that commonly available analogs may be used.

As demonstrated in the accompanying Examples (see Examples 4-6) it has been found that a combinatorial effect is achieved when compounds of the invention are administered together with vitamin D. Accordingly, the invention also encompasses the above methods, further comprising the co-administration of vitamin D, with one or more

compounds of the invention. Other compounds which may be co-administered include aminosterol type compounds; isoleucine or active isomers or analogs thereof; vitamin D type compounds.

Also provided are pharmaceutical compositions comprising, in addition to one or more of the compounds of the invention, vitamin D or one of the other aforementioned compounds as a further ingredient. Such compositions can be formulated in any of the above mentioned formulations and dosage forms.

Oral dosage forms are preferred, as described below.

Preferred dosages

In the methods and compositions of the present invention, the active compound is administered/present in an amount which is effective to stimulate and/or activate this system. Such amount is also referred to herein as a "secretagogue-effective" amount, where the term secretagogue refers to a substance which increases the levels of active antimicrobial peptides in epithelial surfaces.

As noted hereinbefore, PBA has previously been marketed for treatment of hyperammonaemia related to hereditary urea cycle disorders. According to the SPC of Buphenyl (tablet or powder) the drug is dosed at 9.9 to 13.0 g/m^2/day divided into three portions. This amounts to 16 – 23 g daily, or ca. 5.5 to 8.0 g three times daily.

In different studies, topical dosages for PBA used in various studies ranged from 528 mg/day to 1.12 g/day, which corresponds to 35-60% of the normal daily intracolonic production of butyrate. None of these studies reported any adverse effect or reactions. According to one study, daily oral dose of 4g of sodium butyrate given as colonic-targeted tablets for 6-weeks in IBD patients and was also found safe and well tolerated without any adverse effects.

Rabbit studies performed at ICDDRB in Dhaka (see below) showed that dosing about 7.5-22.5 mg/kg was sufficient for therapeutic effect in shigellosis. Scaling this dose to a 70 kg human suggests that a maximally 720 mg daily dose would be effective for the treatment of, for example, shigellosis.

Based on these examples it will be appreciated that a practical upper limit for treatment would be of the order of 20 g/daily (based on urea cycle treatment) and the lower limit may be expected to be lower than 700 mg, e.g. equal to or around 600, 500, 400, 300, 200, 100 mg daily. Potentially even lower amounts may be utilised e.g. 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mg.

It will nevertheless be understood that the suitable amount of the compound to be administered can vary depending on the selected specific compound(s), the specific location of the infection and condition(s) to be treated and/or prevented. In some embodiments, the amount to be administered can be in the range of about 10 µg to about 25 g. A suitable dosage form can be selected and formulated accordingly. For example, for treatment of diseases and conditions in the gastro-intestinal system a dose in the range of 250 µg to about 25 g may be suitable, including the range of about 1 g to about 25 g, e.g. in the range of about 1 g to 10 g, such as about 1 g, 2 g, 5 g or 10 g.

All dosages may be split or given e.g. 1, 2 or 3 times daily.

Administration and formulation

Preferably, the medicament is administered orally but other administration routes are within the scope of the invention and may be more suitable for certain conditions. Such other administration routes include topical, buccal nasal, parenteral, including rectal and vaginal administration.

Inhaled dosage forms include aerosol, inhaler & metered dose inhaler. Ophthalmic dosage forms include eye drops (solution or suspension), ophthalmic gels, and ophthalmic ointments. Otic dosage forms include ear drops (solution or suspension). Rectal dosage forms include enema and suppository. Vaginal dosage forms include douches and pessaries (vaginal suppositories) and vaginal tablets.

Examples of suitable formulations for topical use include creams, ointments, gels, or aqueous or oily solutions or suspensions. Parenteral administration can be accomplished for example by formulating the compound as a sterile aqueous or oily solution for intravenous, subcutaneous, or intramuscular dosing or as a suppository for rectal dosing.

Compositions for oral use may be in the form of hard gelatin capsules in which the active

ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

The compositions can be formulated in various suitable forms, depending on which conditions they are primarily aimed at. In certain embodiments, the compositions are for oral administration. Such compositions include but are not limited to tablets, capsules, a solution, a suspension, a powder, a paste, an elixir, or a syrup.

Compositions may be delayed-release or colonic-targeted compositions such as are well known in the art.

Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions

Another aspect of the invention relates to a pharmaceutical composition for treating, preventing or counteracting any of the above mentioned conditions or diseases. The compositions comprise at least one of the compounds described herein together with at least one pharmaceutically acceptable excipient.

The oral composition of the invention may be formulated for delayed and/or extended release and may be enteric coated by means well known to the skilled person, to be released in the lower intestinal tracts.

Functional foods

It will also be appreciated, in particular when it is desired to administer a large amount of active compound, such as, in the range of 1-25 g that the compounds of the invention can be (isolated and then) formulated and comprised in functional food or feed products. Such functional food products include but are not limited to fermented food products including fermented bean products, e.g. soy bean products such as tempeh, products from fermented oat, germinated barley, and similar products. Such products, generally produced by microbial fermentation which breaks down betaglucans, will have a natural content of short chain fatty acids that can boost the effect of the compounds of the present invention. The form of functional food product in accordance with the invention can be any form suitable for the chosen food type, including crackers, pastry, spread or paste, a purée, a jelly, a yoghurt, a drink concentrate, or any other suitable food product in which the selected active compound(s) can be readily formulated in.

Other species

The methods and compositions of the present invention have application in the treatment of both humans as well as other animals, including veterinary and animal husbandry applications for companion animals, farm animals, and ranch animals. These applications include but are not limited to treating, preventing or counteracting diseases and conditions in dogs, cats, cows, horses, deer and poultry including hen, turkey ducks, geese; as well as in household pets such as birds and rodents. For large animals, a suitable dose can be larger than the above mentioned amounts.

Any sub-titles herein are included for convenience only, and are not to be construed as limiting the disclosure in any way.

The invention will now be further described with reference to the following non-limiting Figures and Examples. Other embodiments of the invention will occur to those skilled in the art in the light of these.

The disclosure of all references cited herein, inasmuch as it may be used by those skilled in the art to carry out the invention, is hereby specifically incorporated herein by cross-reference.

Figures

Figure 1: Fold-induction of *CAMP* mRNA (encoding LL-37) levels in lung epithelial cells (VA10), upon treatment with different agents of the invention. Column c represents a control (untreated cells), Column 3 represents a positive control of vitamin D3 (1,25-dihydroxyvitamin D_3 or 1,25(OH)2D_3) treated cells, column 1 is sodium butyrate and column 2 is sodium 4-phenylbutyrate treated cells. Cells were harvested 24 hours after sodium 4-phenylbutyrate and vitamin D stimulation, and mRNA was isolated. Real time reverse transcription PCR results show how expression of the human cathelicidin gene is affected by sodium 4-phenylbutyrate and vitamin D treatment.

Figure 2: Induction of *CAMP* mRNA expression by butyrate (BA) and PBA derivates. *A)* Structures of utilized chemicals butyrate (BA) 4 mM, 4-phenyl butyrate (PBA) 4 mM, α-methyl hydrocinnamate (ST7) 4 mM, and 2,2-dimethyl-butyrate (ST20) 4 mM. *B)* Induction of *CAMP* mRNA expression by indicated chemicals for 24 hours.

Figure 3: Induction of *CAMP* gene mRNA expression by PBA. *A)* VA10 cells were stimulated with the indicated concentrations of PBA or solvent (Control) for 24 hours. *B)* VA10 cells were stimulated with 4 mM PBA or treated with solvent alone and harvested after the indicated period of time. C) A498, HT-29 and U937 cells were stimulated with 4 mM PBA or solvent only and harvested after the indicated period of time. *CAMP* mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. The normalized data is plotted as mean + SE from at least three independent experiments.

Figure 4: Combinatorial effects of vitamin D and sodium 4-phenylbutyrate stimulation on *CAMP* mRNA expression in lung epithelial VA10 cells, determined as described above for Figure 1. The columns are as follows: C = control; 1 = sodium 4-phenylbutyrate alone; 2 = vitamin D alone; 3 = treatment of sodium 4-phenylbutyrate together with vitamin D.

Figure 5: Further demonstrations of synergetic induction of *CAMP* mRNA and pro-LL-37 expression by PBA (4 mM) and 1,25$(OH)_2D_3$. (20 nM) *A)* VA10 cells were stimulated with PBA (4 mM), 1,25$(OH)_2D_3$ (20 nM) or solvent (Control) for 24 hours. *CAMP* mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Normalized data is plotted as mean + SE from three independent experiments. The differences observed are significant ($P < 0.05$). *B)* VA10

cells were stimulated with PBA (4 mM), $1,25(OH)_2D_3$ (20 nM) or solvent (Control) for 24 hours. Total cell lysates and supernatants analyzed by Western blot for LL-37. One representative blot out of three is shown.

Figure 6A: Induction of the gene encoding LL-37 with sodium 4-phenylbutyrate and vitamin D is affected by the inhibitor U0126 which inhibits the MEK/ERK kinase pathway. C = control; 1 = sodium 4-phenylbutyrate alone; 2 = vitamin D alone. The open columns represent treatment with the inhibitor U0126. The black columns show treatment without the inhibitor. This indicates that the signaling pathways are affected differently by vitamin D and phenylbutyrates.

Figure 6B. Further demonstration of inhibition of PBA induced CAMP gene expression by MAP kinase inhibitors as shown in the Figure, VA10 cells were treated with 4 mM PBA in the presence or absence of 20 μM of the indicated inhibitors. *CAMP* mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Normalized data is plotted as mean + SE from three independent experiments. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Figure 7: Immunohistochemistry showing that CAP-18 (the rabbit homologue to LL-37) is expressed in surface epithelial cells of healthy rabbits, that Shigella infection results in downregulation of the peptide and that this downregulation can be counteracted by oral intake of tributyrylglycerol.

Figure 8: Inhibition of PBA induced CAMP gene expression by cycloheximide shows that translation is necessary. VA10 cells were treated with 4 mM PBA or butyrate (BA) in the presence or absence of 20 μg/ml cycloheximide. CAMP mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression incontrol samples (solvent) where controls were given the arbitrary value of one. Normalized data is plotted as mean plus standard error of the mean from at least three independent experiments. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Figure 9: VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24 hours. Acetylation of histone H3 and H4 was analyzed by quantitative ChIP using antibodies against the respective acetylated histones. Results were normalized to normal

rabbit IgG and total input and plotted as fold precipitation over IgG. Normalized data is plotted as mean + SE from independent experiments (n=3). No significant differences were observed in acetylation of histones.

Figure 10: PBA induced expression does not involve the co-activators of VDR. VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24 hours. mRNA levels of the respective VDR co-activators were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Data is normalized to control and plotted as mean + SE from three independent experiments.

Figure 11: Induction of hBD-1 mRNA expression by PBA. VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24 hours. hBD-1 mRNA levels were determined by real time RT-PCR, *CAMP* induction shown for comparison. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Data is normalized to control and plotted as mean + SE from at least three independent experiments.

Figure 12: Schematic illustration of proposed mechanism for action of PBA treatment in Shigella infected epithelia.

Examples

Example 1

LL-37 expression in lung epithelial cells treated with different agents

Lung epithelial cells (VA 10) were grown to confluency under standard conditions and the agents to be tested added at the indicated concentrations (see below). mRNA was isolated 24 hours after treatment and measured by real time reverse transcription PCR.

Results are shown in Figure 1, where column C represents control (untreated cells), column 3 represents a positive control of vitamin D3 (1,25-dihydroxyvitamin D_3 or 1,25(OH)2D3) (100 nM) treated cells, column 1 is sodium butyrate (2 mM) and column 2 is sodium 4-phenylbutyrate (2 mM) treated cells.

The results show that sodium 4-phenylbutyrate is a more effective inducer of LL-37 mRNA expression than butyrate or vitamin D in VA10 cells, but does not have does not

have the foul smell associated with butyrate. Prior to our studies there were no compounds known to induce LL-37 to the same degree as butyrate let alone without the smell and taste problem. It is particularly surprising that the the deviation from the structure of butyrate can be as substantial as adding an aromatic ring (i.e. doubling the molecular weight). In the light of the present disclosure it may therefore be concluded that that butyrate derivatives, such as aromatic derivatives, will also be active.

In a further experiment, the ability of two other PBA analogs to induce CAMP gene expression was tested (see Figure 2). VA10 cells were stimulated with 4 mM of α-methylhydrocinnamate (ST7), a PBA analog or 2,2-dimethylbutyrate (ST20), a butyrate analog. After 24 hours of incubation, total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. ST7 significantly increased CAMP mRNA expression, while ST20 stimulation had no apparent effect on CAMP mRNA expression levels. Thus it can be seen that quaternary carbon atoms, at least proximal to the carboxyl group, would appear to be undesirable. Conversely, in aryl-butyrate derivatives, it appears that analogs including different chain or branched chains, remain active.

Real time PCR

Six-well plates were seeded with 1.0 x 10^6 cells per well and grown for two days. Medium was then exchanged and different wells were left untreated, supplemented with 2 mM sodium butyrate or 2 mM sodium 4-phenylbutyrate. The cells were incubated for 48 h and total RNA was prepared using the RNEasy kit (Qiagen). Total RNA concentrations were measured using the Quant-iT RiboGreen RNA assay kit (Invitrogen). Superscript III first-strand synthesis system (Invitrogen) was used to synthesize cDNA using random primers according to the protocol of the manufacturer. The expression of the *CAMP* gene, encoding LL-37 was analyzed on the 7500 Real Time PCR System (Applied Biosystems) using the fluorescent probe (5′-6-FAM -TGTTATCCTTATCACAACTGAT-3′ with MGB quencher) and forward and reverse primers specific for the CAMP cDNA (5'-ACCCAGCAGGGCAAATCTC-3' and 5'-GAAGGACGGGCTGGTGAAG-3', respectively). Results were normalized to total RNA quantity, presented as relative fold induction of untreated control cells.

Example 2

LL-37 expression in lung epithelial cells treated with different dose of sodium 4-phenylbutyrate

Figure 3 shows the dose-response of *CAMP* mRNA expression in VA10 lung epithelial cells upon treatment with increasing concentrations of sodium 4-phenylbutyrate. To determine time and dose dependence of PBA induced expression of CAMP mRNA, VA10 cells were stimulated with 4mM PBA over different time points and with different concentrations for 24 hours. Total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. Increase of CAMP mRNA expression was dependent on PBA dose and increased over time.

In earlier experiments it appeard that at higher concentrations, which were non-physiologically relevant (8 mM) the response ceased to be dose-dependent (results not shown).

In earlier experiments in which controls were not measured at the relevant time points, artefacts were seen after long incubations (48 hours; results not shown). Therefore in the experiment shown, controls were measured at the relevant time point and normalised to 1.

The example indicates that successful treatment can be envisaged with a once-daily dosage regimen.

Example 3

Induction of CAMP gene expression by PBA in other cell lines

In order to investigate the effect of PBA on other cell lines, HT-29 (Human colonic adenocarcinoma cell line), A497 (Human renal carcinoma cell line) and U937 (Human leukemic monocyte lymphoma cell line) were stimulated with 4 mM PBA for 8, 24 and 48 hours. Total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. CAMP mRNA expression was signifcantly increased in all cell lines tested (Figure 3C).

Example 4

Synergistic effects of sodium 4-phenylbutyrate and vitamin D on LL-37 expression in lung epithelial cells

A further test shows that sodium 4-phenylbutyrate and vitamin D have combinatorial effects on *CAMP* mRNA expression. VA10 lung epithelial cells were grown as before and treated with sodium 4-phenylbutyrate alone at 2 mM vitamin D alone at 100 nM, and both together, at 2 mM and 100 nM respectively. Treatment with butyrate (at 2 mM) was included as control. Cells were harvested at different timepoints and mRNA was isolated and analysed with real-time reverse transcription PCR.Treatment with both sodium 4-phenylbutyrate and vitamin D clearly show combinatorial effects on mRNA expression level as the effects of the combination are 6-fold higher than of either chemical alone.

In Figure 4, column c shows *CAMP* mRNA levels in the control (untreated cells), column 1 represents treatment with sodium 4-phenylbutyrate alone, column 2 shows treatment with vitamin D alone, and column 4 shows the treatment of sodium 4-phenylbutyrate together with vitamin D.

This is further shown in Figure 6A and 6B. VA10 cells were incubated with a low dose of 20 nM of $1,25(OH)_2D_3$ and 4 mM PBA together and with the respective compounds alone. Expression of CAMP mRNA was found to be higher than the added fold induction of PBA and $1,25(OH)_2D_3$, indicating a synergistic effect (Figure 5).

Example 5

Stimulation by sodium 4-phenylbutyrate and vitamin D acts through different signaling pathways

Epithelial lung cells were treated with sodium 4-phenylbutyrate or vitamin D. For each agent two samples were treated, with and without MAP kinase inhibitor U0126 (concentration of 20 μM) which is specific for inhibiting MEK1 and MEK2 protein kinases.

Results are shown in Figure 6A, where column C represents control (untreated cells), column 1 shows treatment with sodium 4-phenylbutyrate at 2 mM, and column 2 shows treatment with vitamin D (100 nM) for 24 h. The open columns represent treatment with the MAP kinase inhibitor U0126, whereas the black columns show treatment without the inhibitor.

The results shown indicate that different signaling pathways are involved in the induction by sodium 4-phenylbutyrate and vitamin D; this may explain the combined effects of the chemicals on the induction of the *CAMP* gene.

The effect of inhibitors for c-Jun N-terminal kinase (JNK), p38 kinase and extracellular signal-regulated kinase 1/2 (ERK1/2) on PBA induced CAMP gene expression were also investigated as shown in Figure 6B. One hour prior to stimulation with 4 mM PBA, VA10 cells were pre incubated with 20 μM SP600125, SB203580 or U0126 to inhibit the respective kinases. After 24 hours of incubation, total RNA was isolated and analyzed by real time RT-PCR for CAMP mRNA. Inhibitors for the ERK1/2 and JNK pathways significantly reduced PBA induced CAMP gene expression.

Example 6

Shigella infected rabbits treated with glyceryl tributyrate

It has been confirmed by immunohistochemistry that CAP-18 (the rabbit homologue to LL-37) is expressed in surface epithelial cells of healthy rabbits (Figure 7A) and that Shigella infection results in downregulation of peptide production (Figure 7B). Furthermore, upon treatment with tributyrylglycerol, the downregulation of gene expression by Shigella is reverted and/or prevented (Figure 7C).

Animal model: Inbred New Zealand White rabbits of either gender weighing 1.8 to 2 kg were used for the study. The animals were individually caged in a room maintained at 22-25°C. Before inclusion in the study, health status of the rabbits was determined by physical examination, culture of stool and rectal swab specimens and fecal parasitic examination. Healthy coccidia-free rabbits that were also free of enteric pathogens (e.g. *Salmonella, Shigella, Vibrio cholera*) were studied. Rabbits were infected with *Shigella* and divided into two groups, one group was treated orally with glyceryl tributyrate and the other with saline. Expression of the CAP-18 peptide and its proform in colonic and rectal tissue specimens were analyzed in healthy rabbits, in untreated infected rabbits, in infected and healthy rabbits treated with glycerol tributyrate. For analyses of toxicity effects of glycerol tributyrate healthy rabbits were also treated with this compound.

Bacterial strain and inoculum preparation: The *Shigella flexneri* 2a strain was isolated from stool of a patient. The strain was positive for the Serény test and Congo red binding, reflecting invasive properties (Berkhoff, H.A. and Vinal, A.C., 1986, Avian Dis. 30, 117-

121)) From this stock, bacteria were subcultured on trypticase soya agar (TSA; Becton Dickinson, Sparks, MD) plates and cultured overnight at 37°C. Three to five smooth colonies were inoculated in trypticase soya broth and cultured for 4 h with shaking at 37°C. The broth was then washed in normal saline at 7000 rpm for 10 min and bacterial pellet was suspended in normal saline to a concentration of 1 x 10^9 cfu in 7 mL that were given to the rabbits.

A non-surgical rabbit model of shigellosis was used in this study as described previously with slight modifications (Etheridge, M.E. et al., 1996, Lab. Anim. Sci. 46, 61-66). Briefly, rabbits were fasted for 36 hours and given a single oral dose of a tetracyclin hydrochloride (250 mg/kg; Novartis, Dhaka, Bangladesh) suspension. After that, rabbits were anesthetized with sodium pentobarbitol (33 mg/kg; Sigma, Chemical Co, St Louis, MO) and given 37.5 mg/kg weight of G-cimetidine (Gonoshasthoya Pharmaceuticals, Dhaka, Bangladesh) intravenously via the marginal car vein to inhibit gastric secretion. Fifteen minutes later, 7 ml of 5% sodium bicarbonate solution was administered orally with a sterile plastic feeding tube (3.33 x 465 mm, Tycohealthcare Ireland Ltd., Tullamore, Ireland), which was followed 15 minutes later by a second 15-ml dose of 5% sodium bicarbonate solution and a 7-ml dose of the bacterial suspension (10^9 cfu in 7 ml normal saline (0.9% w/v, pH 7.2)) immediately thereafter. Twenty minutes after inoculation of the bacterial suspension, 7 ml of Loperamide HCl (0.02 mg/kg body weight) in normal saline was introduced orally to reduce intestinal motility. Thereafter, rabbits were allowed to eat and drink regular food. Usually rabbits developed dysentery within 24 hours of bacterial inoculation. Time of bacterial inoculums was considered as 0 hr. After development of dysenteric symptoms, rabbits were given glyceryl tributyrate (47 µmol/kg body weight, i.e., 140 µmol butyrate equiv./kg) by an orogastric feeding tube twice daily at twelve hours interval for 3 days. Four days after bacterial inoculation, rabbits were given an overdose of intravenous sodium pentobarbitol (66 mg/kg; Sigma) for euthanasia.

To evaluate the presence of the CAP-18 peptide immunohistochemical staining was performed by using the chicken polyclonal antibody specific to CAP-18 (Innovagen). Briefly, paraffin sections were deparaffinized, hydrated and given microwave treatment in retrieval buffer (Dako laboratories A/S, Glostrup, Denmark) for 12 minutes followed by washing in phosphate buffer (pH 7.2). After cooling, endogenous peroxidase activity was quenched and sections were incubated overnight with the CAP-18-specific antibody (2 µg/ml) at room tempture. After washing, sections were incubated with horse-radish-peroxidase conjugated donkey anti-chicken antibody (1:200; Jackson ImmunoResearch

Laboratories, Inc.) for 1 hr at room temperature. This was followed by washing and development of the color was with diaminobenzidine (DAB, brown). As a control, specific antibodies were replaced by irrelevant isotype-matched-antibodies. In addition, synthetic CAP-18 was incubated at 10-fold higher concentration with the CAP-18 antibody overnight at 4°C and the mixture was used as above for immunostaining. This served as control for the specific staining. After counter-staining in hematoxylin and eosin, slides were mounted in paramount (BDH Chemicals, Poole, England).

Clinical recovery of the rabbits from shigellosis was established by disappearance of blood from stool, reappearance of formed stool, normalization of weight, body temperature, return of normal appetite and playful activity.

Example 7

Inhibition of PBA induced CAMP gene expression by cycloheximide

In order to assess whether the PBA and butyrate induction pathways of CAMP gene expression are direct, VA10 cells were treated with PBA or butyrate in the presence and absence of cycloheximide (CHX). After 24 hours of incubation, total RNA was isolated and CAMP mRNA levels measured using real time RT-PCR. Pre-incubating the cells with 20 µg/ml of CHX for one hour prior stimulation effectively blocked both PBA and butyrate induced CAMP gene expression

This suggests that that PBA induced CAMP gene expression is induced through a secondary effect. This secondary induction pathway may depend on MAP kinase signaling through JNK and ERK1/2 as it was shown in VA10, a bronchial epithelial cell line (see Figures 6A and 6B).

Example 8

The effect of PBA on histone acetylation at the CAMP gene promoter

The effect of PBA on acetylation of histone H3 and H4 by quantitative chromatin immunoprecipitation was assessed. No significant change in histone acetylation could be observed at the CAMP gene proximal promoter (1000 bp upstream of transcription start site) after treatment with 4 mM PBA for 24 hours (Figure 9)

Earlier it has been assumed that induction of CAMP gene expression by histone deacetylase inhibitors occurs through an increase of histone acetylation and relaxation of chromatin structure, facilitating the binding of other transcription factors. The present data speaks against this hypothesis. Assessing acetylation of H3 and H4 at the CAMP proximal promoter using quantitative chromatin immunoprecipitation, a significant change in acetylation was detectable after treatment with PBA. Furthermore, it was previously shown (see Example 7) that inhibiting protein synthesis using cycloheximide blocks both butyrate and PBA induced expression of CAMP gene expression. These results rule out that an increase of histone acetylation at the CAMP proximal promoter by these compounds directly facilitates CAMP gene expression. Without wishing to be bound by theory, it is believed that an increase of histone acetylation facilitates the expression of other genes, which then increase CAMP gene expression as a secondary effect.

Example 9

The effect of PBA on vitamin D co-activator expression

Hypothesizing that the synergistic effect between PBA and $1,25(OH)_2D_3$ was due to an induction of VDR co-activator genes by PBA, we analyzed the effect of PBA on mRNA levels of several known VDR co-activator genes in VA10. None of the genes were significantly upregulated after treatment with 4 mM PBA for 24 hours (see Figure 10). These co-activators are therefore not involved in the PBA-induced effects on gene expression.

Example 10

Induction of hBD-1 mRNA expression by PBA

CAMP is not the only antimicrobial defense gene that is induced by PBA. Another well-known peptide is also induced, although at lower level than CAMP (See Figure 11). This suggests that PBA has a general effect on mucosal defenses.

Example 11

Synthesis of glyceryl tributyrate

Butanoic anhydride (164 ml, 1.0 mol) was added during 10 min to glycerol (7.34 ml, 100 mmol) in Pyridine (300 ml) at 0°C. The mixture was stirred at 0°C for 10 min and at room

temperature for 18 h. Water (200 ml) was added and the mixture was heated at 60°C for 15 min. Evaporation of solvent gave a residue that was partitioned between dichloromethane (DCM, 400 ml) and $NaHCO_3$ (20 % in water, 400 ml). The aqueous layer was further extracted with DCM (50 ml). The combined organic extracts were washed first with saturated aqueous $NaHCO_3$ (400 ml) and then with HCl (1M in water, 400 ml). The organic layer was collected and dried with Na_2SO_4 and then concentrated *in vacuo* to afford 29.6 g (98 %) of glyceryl tributyrate ^{1}H NMR ($CDCl_3$), 0.95 (t; *J*=7.4 Hz; 2 X CH_3), 0.96 (t; *J*=7.4 Hz; CH_3), 1.60-1.73 (m; 3 X CH_2), 2.31 (t; *J*=7.4 Hz; 2 X CH_2), 2.32 (t; *J*=7.35 Hz; CH_2), 4.16 (dd + AB; *J*=11.9, 6.0 Hz; 2 X CH_a), 4.31 (dd + AB; *J*=11.9, 4.3 Hz; 2 X CH_b), 5.29 (m; 5.26-5.31; *CH*).

Example 12

Synthesis of N-Butanoylglycine ethyl ester

Glycine ethyl ester hydrochloride (13.96 g, 100 mmol) and triethylamine (34.65 ml, 250 mmol) in dichloromethane (DCM, 500 ml) was stirred for 2 h at room temperature, which resulted in a fine white precipitate. Butanoic anhydride (19.63 ml, 120 mmol) in DCM (100 ml) was added over 5 min and the reaction mixture turned to a clear solution. After 30 min at room temperature, and subsequent removal of solvent (*in vacuo*), water was added (18 ml, 1 mol) followed by pyridine (23.73 g, 24.26 ml, 300 mmol). The solution was heated at 60°C for 30 min. The mixture was partitioned between DCM (200 ml) and aqueous HCl (2.4 M, 200 ml, saturated with NaCl). The aqueous layer was separated and extracted with DCM (50 ml). The combined organic extract was washed with HCl (*aq.*, 1 M, 250 ml) and the water layer was extracted with an additional portion of DCM (50 ml). The combined organic extracts was washed with $NaHCO_3$ (*aq.*, 4.2 %, 200 ml) and the water layer extracted once more with DCM (50 ml). The combined organic extracts was dried with Na_2SO_4 and concentrated *in vacuo* yielding 16.3 g (94 %) of N-butanoylglycine ethyl ester. ^{1}H NMR ($CDCl_3$), 0.97 (t; *J*=7.4 Hz; CH_3), 1.30 (t; *J*=7.1 Hz; CH_3), 1.65-1.74 (m; CH_2), 2.23 (t; *J*=7.5 Hz; CH_2), 4.05 (d; 4.9 Hz; CH_2), 4.23 (q; 7.2 Hz; CH_3), 5.9 (broad; *NH*).

Example 13

Synthesis of N-Butanoylglycine

N-Butanoylglycine ethyl ester (16.3 g, 94.16 mmol) was dissolved in aqueous NaOH (1 M, 282 ml, 282 mmol) and then stirred for 15 h at room temperature. Aqueous HCl (12 M,

15.7 ml, 188 mmol) was added to pH=5. The water was then evaporated (*in vacuo*) and the residue was dissolved in aqueous HCl (1 M, 175 ml) which gave a pH of 1. The solution was saturated with NaCl and extracted with tetrahydrofuran (3 X 100 ml). The combined organic extracts was dried with Na_2SO_4 and evaporated *in vacuo* yielding 13 g (95%) of N-butanoylglycine. ^{1}H NMR ($CDCl_3$), 0.97 (t; *J*=7.4 Hz; CH_3), 1.64-1.74 (m; CH_2), 2.27 (t; *J*=7.5 Hz; CH_2), 4.09 (d; *J*=5.1 Hz; CH_2), 6.24 (broad; *NH*), 8.1 (broad; *COOH*).

Example 14

Synthesis of N^{α},N^{ε}-dibutanoyllysine

Lysine (1g, 6.1 mmol) was dissolved in 160 ml tetrahydrofuran(THF)-water (1:1), whereupon butanoic anhydride (2.89g 18.3 mmol) was added. The solution was kept stirring at room temperature and after 1h 80 ml of THF was added and after standing overnight sodium carbonate decahydrate was added (5.23 g, 18.3 mmol). After this mixture was stirred for ca 30 min another portion of butanoic anhydride (2.89g 18.3 mmol) was added and the mixture was again kept stirring overnight. The mixture was saturated with sodium chloride and made acidic with concentrated HCl (to about pH 1). The top layer was separated and the solvent was evaporated. To the residue 400 ml 0.125 M NaOH (*aq*) and 100 ml THF was added. After ca 15 the THF was evaporated and the solution was washed with chloroform (2x200 ml). The aqueous phase was then acidified with 7 ml conc. HCl (*aq*) and extracted with chloroform-methanol (4:1, 2X250 ml). The organic phase was dried with sodium sulfate, filtered and concentrated under reduced pressure. The remaining butanoic acid was removed by repeated evaporation of added formic acid-water (3:1) under reduced pressure to give 1.32 g (79%) of product. ^{1}H NMR ($CDCl_3$), 0.92-0.98 (m, 6H; 2xCH_3), 1.3-1.48 (m, 2H; CH_2), 1.54 (qv, 2H, *J*=6.8 Hz; CH_2), 1.62-1.70 (m, 4H; 2xCH_2), 1.75-1.83 (m, 2H; CH_2), 1.85-1.95 (m, 2H; CH_2), 2.18 (t, 2H, *J*=7.3 Hz; CH_2), 2.25 (t, 2H, *J*=6.2 Hz; CH_2), 3.17-3.22 (m, 1H; ε-CH_{2a}), 3.31-3.37 (m, 1H; ε-$_{CH2b}$), 4.52-4.58 (m, 1H; α*CH*), 6.08 (bs, 1 H; ε-NH), 6.86 (d, 1 H, *J*=7.3 Hz; α-NH).

Example 15

Demonstration of effectiveness of butyrate-class compounds in human infectious colitis (shigellosis)

The following trial is performed with sodium butyrate enema but may be performed correspondingly using PBA for oral administration.

Requirement of a population

Sodium butyrate enemas have been applied in inflammatory bowel diseases, including ulcerative colitis, diversion colitis, Crohn's Diseases but never in an infectious colitis.

Adult patients with shigellosis have been selected to assess the efficacy in infectious colitis which may be later conducted in children.

Selection of butyrate enema over oral tablets

A large body of evidence is available to show that sodium butyrate enema given over a range of 2 –6 weeks in adult patients with inflammatory bowel disease (IBD) is safe with no obvious side effects. The topical dosage used in various previous studies ranged from 528 mg/day to 1.12 g/day, which corresponds to 35-60% of the normal daily intracolonic production of butyrate. None of these studies reported any adverse effect or reactions. According to one study, daily oral dose of 4g of sodium butyrate given as colonic-targeted tablets for 6-weeks in IBD patients and was also found safe and well tolerated without any adverse effects. The present study utilised enema.

Study design: A double blind randomized clinical trial with subsequent follow-up.

Study Subjects: Adult male and female patients attending the Dhaka Hospital and Matlab Hospital of ICDDR,B are screened for participation in the study.

Inclusion criteria:

- 18-45 years of age
- Males & females
- duration of diarrhoea 0-3 days
- culture-confirmed *Shigella spp* (all Shigella *spp*) in stool on enrolment

Exclusion criteria:

- who received antimicrobial treatment before attending the ICDDR,B hospital
- clinical symptoms of other concomitant infections (such as chronic respiratory infections, other concomitant gastrointestinal infections)

Randomization

According to a computer-generated randomization list, patients full filling the entry criteria is randomized to either intervention group (Pivmecillinam plus butyrate enema) or control/placebo group (Pivmecillinam plus normal saline enema).

Composition of enema and procedure for enema

Butyrate enema contains 80 mmol/L of butyrate in normal saline (pH 7.2).
Placebo enema contains 30 mmol/L NaCl (pH 7.2).

The patient is instructed to lie on a bed (cholera cot) in left lateral position. A soft rectal catheter is introduced by a nurse/physician, through which 80 ml of butyrate solution is instilled slowly with a 50 ml plastic syringe. The patient is asked to retain the enema for at least ½ hour by remaining supine for 30 minutes after the administration. However, if a patient cannot retain the enema for 30 minutes, he is given a second round of enema immediately after defecation.

Case Management

After enrolment, the patients are admitted in the study ward of ICDDRB Dhaka and Matlab hospital. A standard clinical history and clinical examination is performed by the study physician. All patients receive Pivmecillinam, 400 mg, 8 hourly for 5 days. The intervention group receives butyrate enema 80 ml of 80 mM sodium butyrate, 12 hourly for 72 hours while the placebo group gets 80 ml of normal saline 12 hourly for 72 hours. All patients receive the usual hospital food three times a day (breakfast, lunch and supper). The patients remain in the study ward for 5 days to enable identification of any relapse cases.

Sample size

In a study by Kabir I et al (1984) (*Kabir I, Rahaman MM, Ahmed SM, Akhter SQ, Butler T. Comparative efficacies of pivmecillinam and ampicillin in acute shigellosis. Antimicrob Agents Chemother. 1984 May;25(5):643-5.*), it has been shown with 3.2 ± 1.8 (mean ±SD) duration of diarrhoea of patients with shigellosis while treated with pivmecillinam. Expecting a 30% reduction in duration of diarrhoea when treated with butyrate enema along with pivmecillinam, considering 5% level of significance and 80% power the sample size will be 55 per group. Considering a dropout of 10%, the sample size in each group will be 61.

Clinical Parameters measured / recorded

1. Appetite
2. Abdominal cramps
3. Rectal tenesmus
4. Body temperature, 8 hourly
5. Daily frequency of stool (No. of times of defecation)
6. Stool output (in grams)
7. Presence of RBC, pus cells and macrophages in stool by RME
8. Weight at admission, daily during hospitalization and after 14 days (at follow-up)
9. Sigmoidoscopic findings

Other analysis

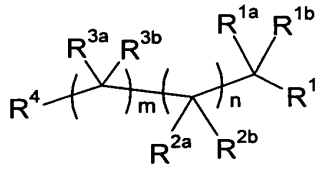
1. Stool culture by serial dilution method for bacterial count (twice daily) for 4 days.
2. Stool for detection of LL-37 by Western blot
3. Stool for determination of LL-37 by ELISA
4. Rectal biopsy (from Dhaka patients only) for histologic grading of inflammation.
5. Rectal biopsy for immunohistochemical staining of LL-37 and image analysis.
6. Rectal biopsy for assessing transcripts of LL-37 in tissue by realtime PCR.
7. Serum for measuring butyrate

Data analysis

For normally distributed data, it is intended to use appropriate parametric tests (eg. t test) to compare the results between groups. In case the data is skewed, nonparametric tests will be used. Statistical analysis can then be done using two-factor ANOVA to determine significant interactions between time and treatment and in case of any significant interactions post hoc Tukey procedure will be performed. For data that are not normally distributed, ANOVA on ranks will be applied. For within group (between days) comparisons, one-way ANOVA will be done. Statistical calculations will be performed using the statistical software SigmaStat® 3.1 (Jandel Scientific, San Rafael, Calif.) and SPSS 13.

CLAIMS

1. A compound of formula Ia for use as a medicament for treating, counteracting or preventing microbial infection in an animal by stimulating the innate antimicrobial peptide defense system:



(Ia)

wherein

R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof;

m and *n* are each independently 0 or 1;

R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a} and R^{3b} each independently represent hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group; and/or

R^{2a}, together with an adjacent R^{3a} or R^{1a}, may represent a carbon-carbon π bond; and/or

R^{2b}, together with an adjacent R^{3b} or R^{1b}, may represent a carbon-carbon π bond;

R^4 may be hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group;

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group;

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group; and

R^7 is a side chain of a naturally occurring amino acid or is selected from $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, or $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms;

and wherein, if R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is selected from halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group.

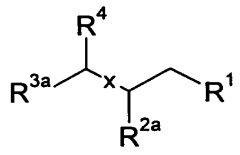
2. A compound as claimed in claim 1 wherein:

R^{1a} and R^{1b} are both hydrogen,
m and *n* are both 1, and R^{2b} and R^{3b} are *either* both hydrogen *or* together form a π bond in position 'x' whereby if R^{2a} and R^{3a} also together form a π bond, then position 'x' represents a double bond,

or wherein:

R^{1a}, R^{1b} and R^{2b} are all hydrogen,
m is 0, *n* is 1,
and R^4 is hydrogen,

such that the compound has formula I.



wherein

R^1 represents a carboxyl group, phospate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

x represents a single, double or triple bond,

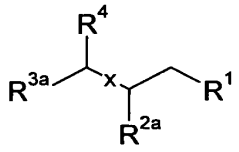
or $x\text{-}R^{3a}R^4$ together represent hydrogen in which case R^1 is $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CNHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms.

3. A compound of formula I for use as a medicament for treating, counteracting or preventing microbial infections in an animal, including humans, by stimulating the innate antimicrobial peptide defense system



I

wherein

R^1 represents a carboxyl group, phospate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

x represents a single, double or triple bond,

or x-$R^{3a}R^4$ together represent hydrogen in which case R^1 is $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CNHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms.

4. The compound of any one of claims 2 to 3 wherein R^1 represents a carboxyl group or a pharmaceutically acceptable salt thereof.

5. The compound of any one of claims 2 to 4 wherein R^1 represents an ester group of formula $COOR^5$.

6. The compound of any one of claims 2 to 5 wherein R^{2a} and R^4 represent hydrogen.

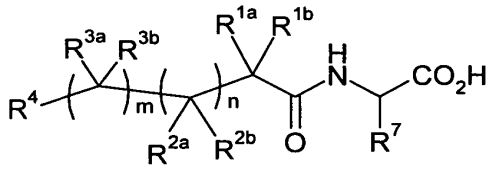
7. The compound of claim 6, wherein R^{3a} represents a substituted or nonsubstituted aryl group.

8. The compound of any of the aforementioned claims wherein R^5 and R^6 independently represent a linear or branched acyl chain with three to five carbon atoms.

9. The compound of claim 1 wherein at least one of *m* and *n* is 1, R^1 represents a carboxyl group or a pharmaceutically acceptable salt thereof and at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen, or R^1 is a carboxylic acid derivative selected from: ester, amide.

10. The compound of claim 9 wherein R^1 is an ester selected from a triglyceride ester moiety or diglyceride ester moiety.

11. The compound of claim 9 wherein R^1 is an amide of an amino acid group such that the compound has the general formula (IIIe):



(IIe)

or a salt thereof, in which R^7 is a naturally occuring amino acid side chain.

12. The compound of any one of claims 9 to 11 wherein one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group and the others are selected from hydrogen or an alkyl group.

13. The compound of claim 12 wherein one of R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group and the others are selected from hydrogen or an alkyl group.

14. The compound of any one of claims 9 to 13 wherein at least one of R^{1a} and R^{1b} is hydrogen.

15 The compound of any of claims 1 to 14 wherein R^5 and R^6, if present, are independently represent propanoyl, *n*-butanoyl, or *iso*-butanoyl.

16 The compound of any of claims 1 to 15 wherein R^8, if present, represents propanoyl, *n*-butanoyl, or *iso*-butanoyl.

17 The compound of any of claims 1 to 16 whereinselected from the group consisting of: 4-phenylbutyric acid, 3-phenylbutyric acid, 2-phenylbutyric acid, 3-phenylpropionic acid, 2-phenylpropionic acid, 2-methyl-3-phenylpropionic acid *[ST7]*, 2-methyl-4-phenylbutyric acid, or a pharmaceutically acceptable salt of any of said compounds, methyl 4-phenylbutyrate, ethyl 4-phenylbutyrate, methyl 3-phenylbutyrate, ethyl 3-phenylbutyrate, methyl 2-phenylbutyrate, ethyl 2-phenylbutyrate, methyl 3-phenylpropionate, ethyl 3-phenylpropionate, methyl 2-phenylpropionate, ethyl 2-phenylpropionate, methyl 2-methyl-3-phenylpropionate, ethyl 2-methyl-3-phenylpropionate, methyl 2-methyl-4-phenylbutyrate, and ethyl 2-methyl-4-phenylbutyrate.

18 The compound of any of claims 1 to 17, wherein said microbial infection is selected from the group consisting of bacterial, viral, protozoal and fungal infections.

19 The compound of claim 18, wherein said microbial infection is caused by a microbial species selected from: Yersenia enterocolitica, Salmonella, Shigella, Campylobacter, Clostridium and E. Coli.

20 The compound of any one of claims 1 to 19, wherein said microbial infections results in gastrointestinal disorders selected from the list consisting of: traveller's diarrhoea, endemic diarrhoea, dysentery, viral gastroenteritis, parasitic enteritis, Crohn's disease, ulcerative colitis, irritable bowel syndrome, precancerous states of the

gastrointestinal tract, cancer of the gastrointestinal tract, diverticulitis, post-antibiotic diarrhoea, Clostridium difficile colitis, lactose intolerance, flatulence, gastritis, esophagitis, heartburn, gastric ulcer, ulcers associated with Helicobacter pylori, duodenal ulcer, short bowel syndrome, dumping syndrome, gluten enteropathy and food intolerance; eye infections optionally selected fromconjunctivitis, stye, blepharitis, cellulitis, keratitis, corneal ulcer, trachoma, uveitis, canaliculitis and dacryocystitis; urinary tract and genital infections optionally selected from pyelonephritis, cystitis, gonorrhoea and urethritis; infections of the respiratory system optionally selected from bronchitis, pneumonia, rhinosinusitis, sinusitis, pharyngitis/tonsillitis, laryngitis and influenza; skin infections optionally selected from boils, carbuncles, furuncles, cellulitis, abscesses, impetigo, and erysipelas; infections caused by bacterial strains resistant to classical antibiotic treatment.

21. The compound of any of claims 1 to 20 wherein the microbial infection in the animal has lead to down-regulation of the innate antimicrobial peptide defense system, and whereby stimulation of the innate antimicrobial peptide defense system upto or above basal levels leads to secretion of the relevant peptide onto an epithelial surface which is optionally in the gastrointestinal tract such as to enhance the antimicrobial activity thereof.

22. The compound of any of claims 1 to 21 for use in a combination treatment for treating, counteracting or preventing microbial infection in an animal, wherein the compound is used in combination with any one or more of: an antibiotic; an aminosterol-type compound; isoleucine or active isomers or analogs thereof; a vitamin D type compound.

23. A pharmaceutical composition for treating, preventing or counteracting a microbial infection comprising as an active ingredient at least one compound of any one of claims 1 to 21 and at least one pharmaceutically acceptable excipient.

24 The pharmaceutical composition of claim 23, formulated as an oral dosage form.

25 The pharmaceutical composition of claim 24, wherein said oral dosage form is selected from a tablet, a capsule, a solution, a suspension, a powder, a paste, an elixir, a syrup.

26 The pharmaceutical composition of any one of claims 23 to 25, wherein a unit dose of said composition comprises in the range of about 10-1000 mg of said active ingredient.

27 The pharmaceutical composition of any one of claims 23 to claim 26 further comprising any one or more of: an antibiotic; an aminosterol-type compound; isoleucine or active isomers or analogs thereof; a vitamin D type compound.

28 A functional food or feed product comprising an amount of at least one compound of any one of claims 1 to 21, which amount is effective for treating, counteracting or preventing bacterial infections in an animal being fed with said food or feed.

29 The functional food or feed product of claim 28, comprising in the range of about 0.1 to 20 mg of the active ingredient per g of food product.

30 A method for treating, preventing or counteracting microbial infection in an animal, wherein the effects of the microbial infection are diminished or reduced by upregulation of the innate antimicrobial peptide system, said method comprising administration of a medicament comprising a secretagogue-effective amount of at least one compound of formula I as defined in any one of claims 1 to 22.

31 The method of claim 30, comprising administration of said medicament in an oral dosage form.

32 The method of claim 31, wherein the daily dosage is between 250 µg to about 25 g which is optionally split into doses given 1, 2 or 3 times daily.

33 A compound, composition, food, or method as claimed in any one of the preceding claims wherein the animal is a human.

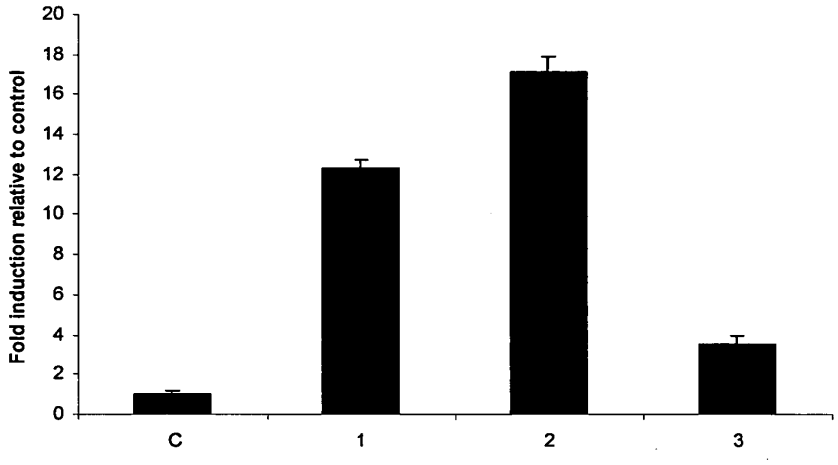

Fold induction relative to control
20
18
16
14
12
10
8
6
4
2
0
C
1
2
3

Figure 1.

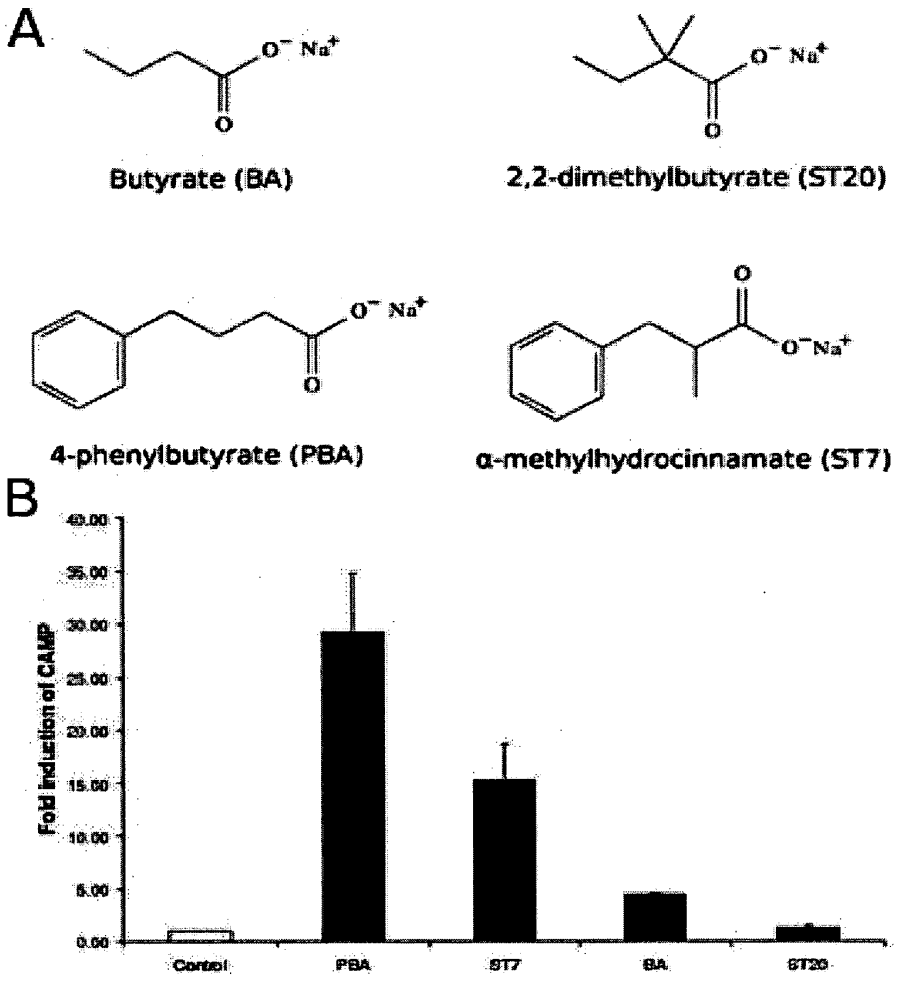

A
Butyrate (BA)
2,2-dimethylbutyrate (ST20)
4-phenylbutyrate (PBA)
α-methylhydrocinnamate (ST7)
B
Fold induction of cAMP
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Control
PBA
ST7
BA
ST20

Figure 2

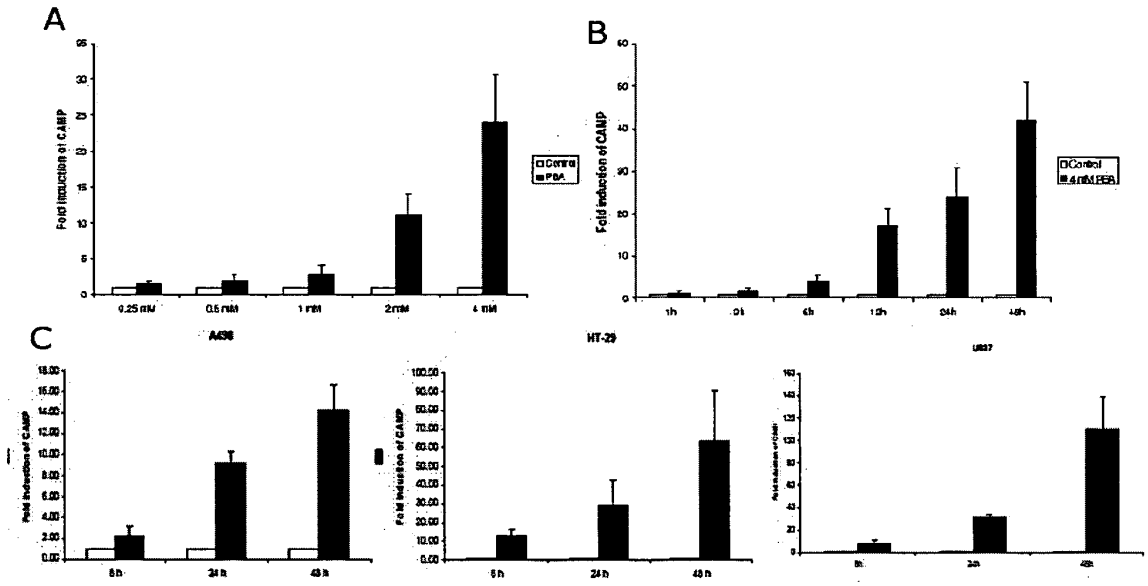


Figure 3

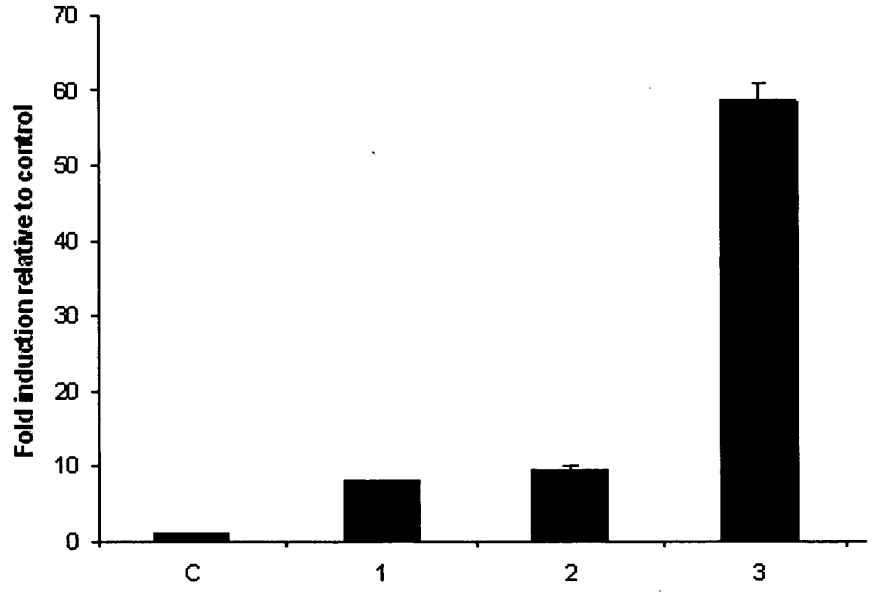


Figure 4

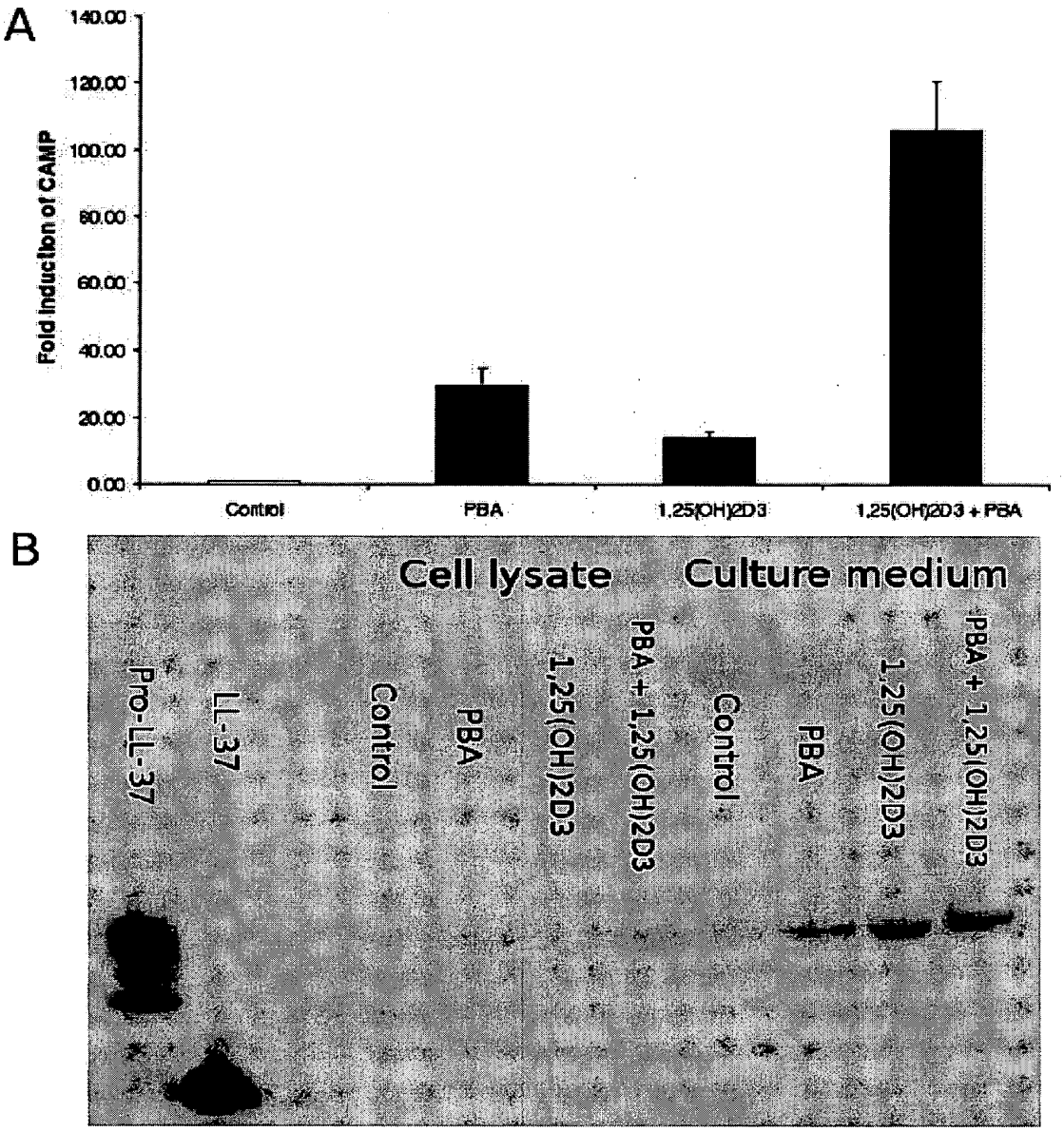
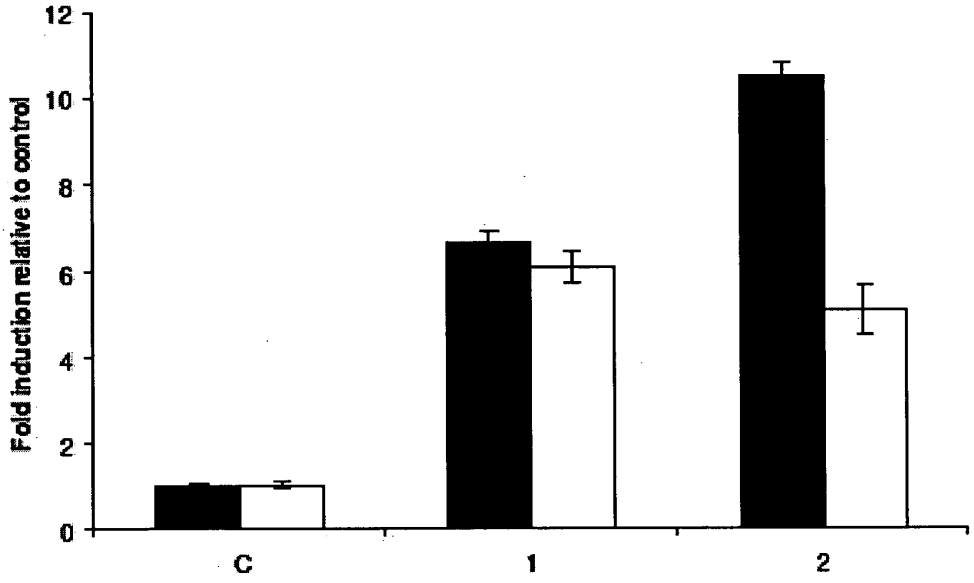

A
Fold induction of CAMP
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Control
PBA
1,25(OH)2D3
1,25(OH)2D3 + PBA
B
Cell lysate
Culture medium
Pro-LL-37
LL-37
Control
PBA
1,25(OH)2D3
PBA + 1,25(OH)2D3
Control
PBA
1,25(OH)2D3
PBA + 1,25(OH)2D3

Figure 5

A


Fold induction relative to control
12
10
8
6
4
2
0
C
1
2

B

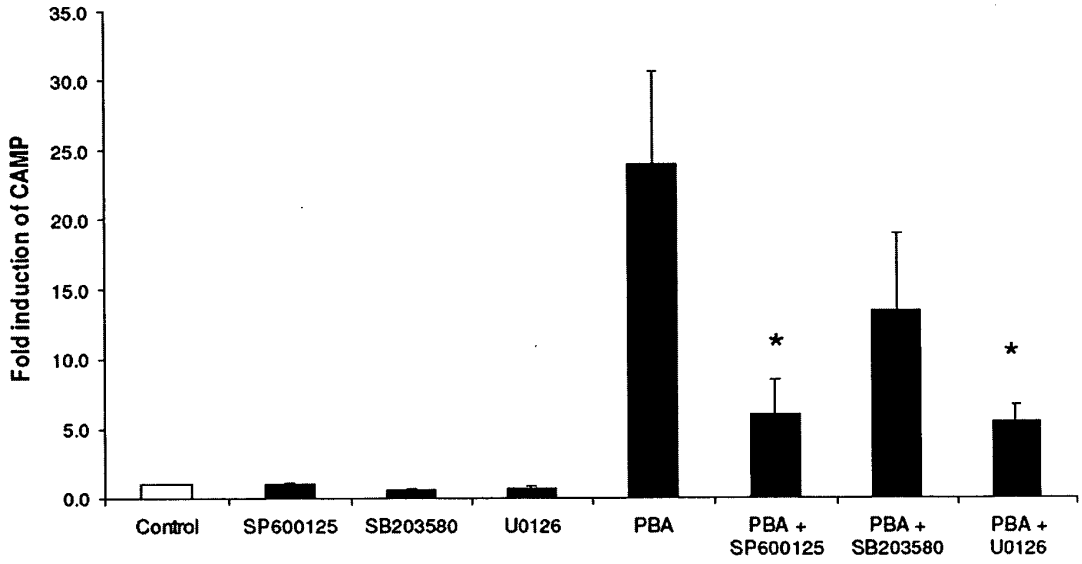

Fold induction of CAMP
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
*
*
Control
SP600125
SB203580
U0126
PBA
PBA + SP600125
PBA + SB203580
PBA + U0126

Figure 6

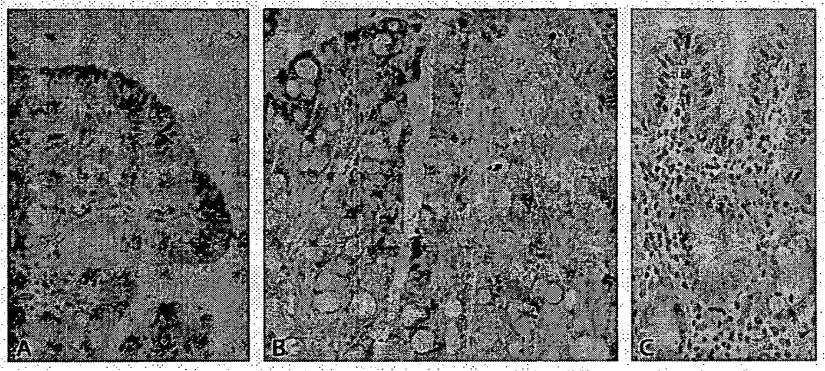


Figure 7

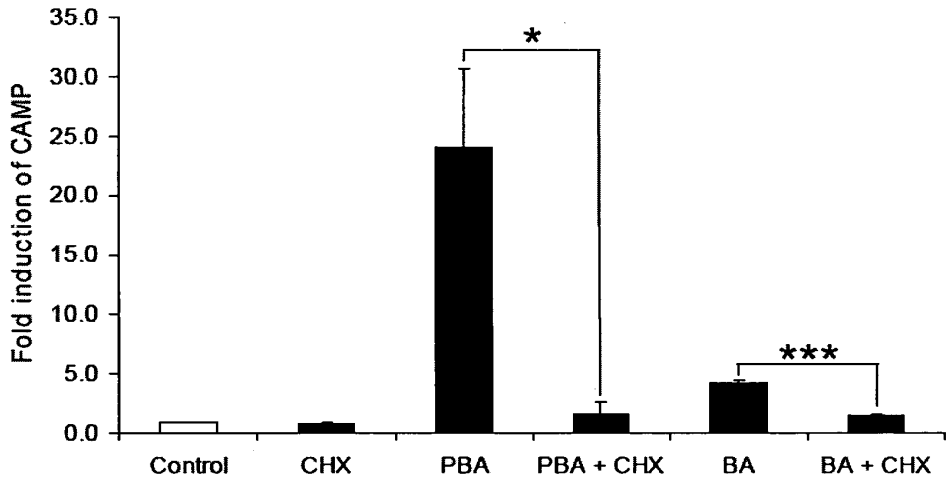


Figure 8

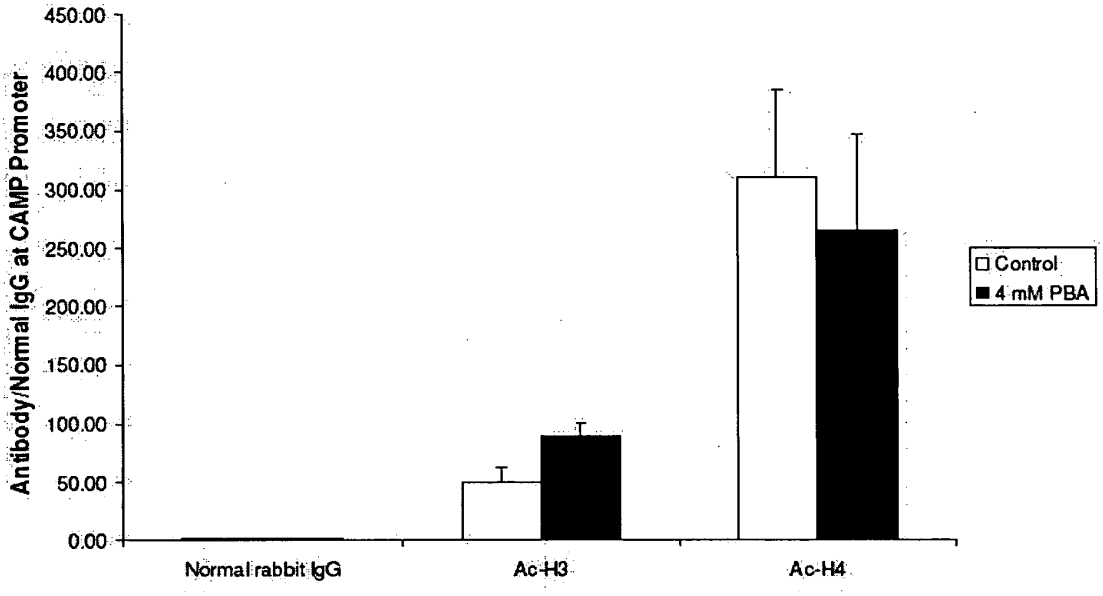


Figure 9

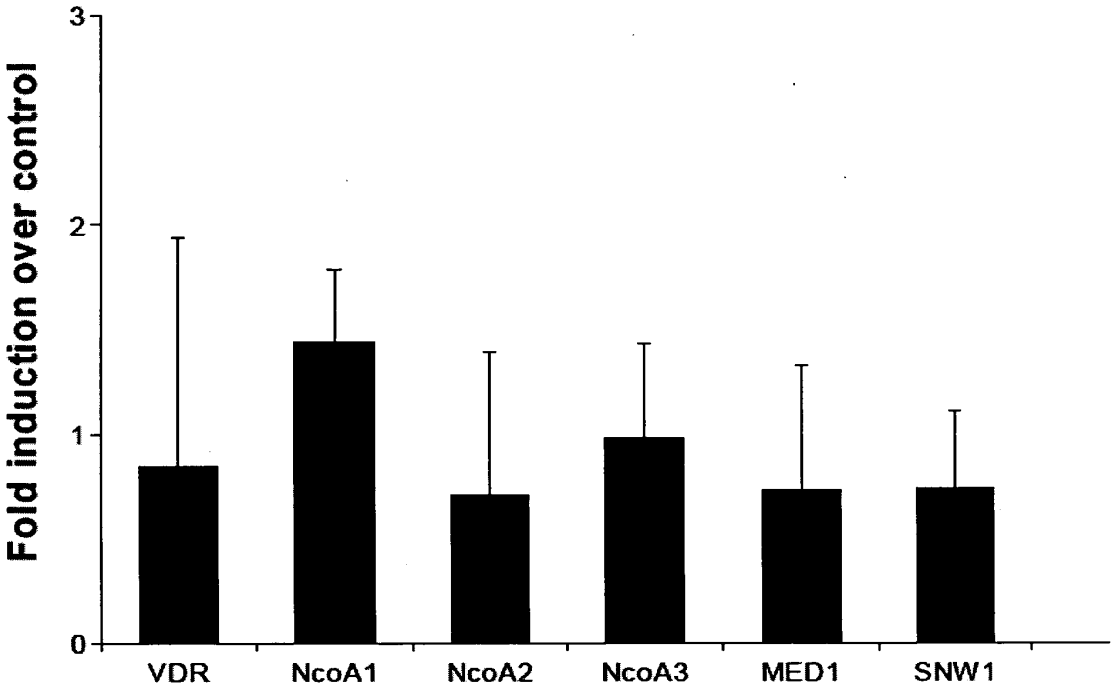


Figure 10

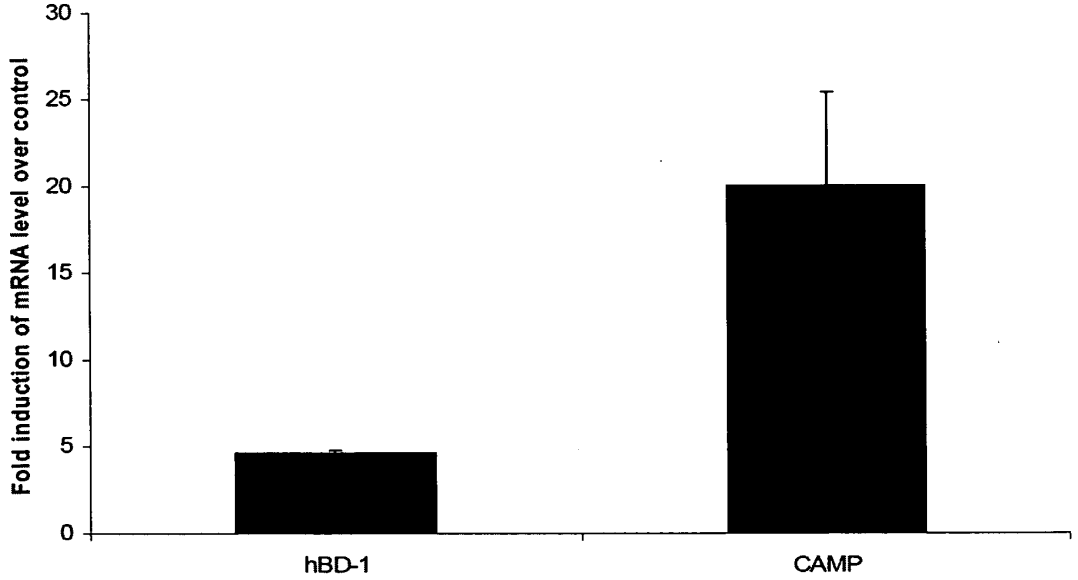
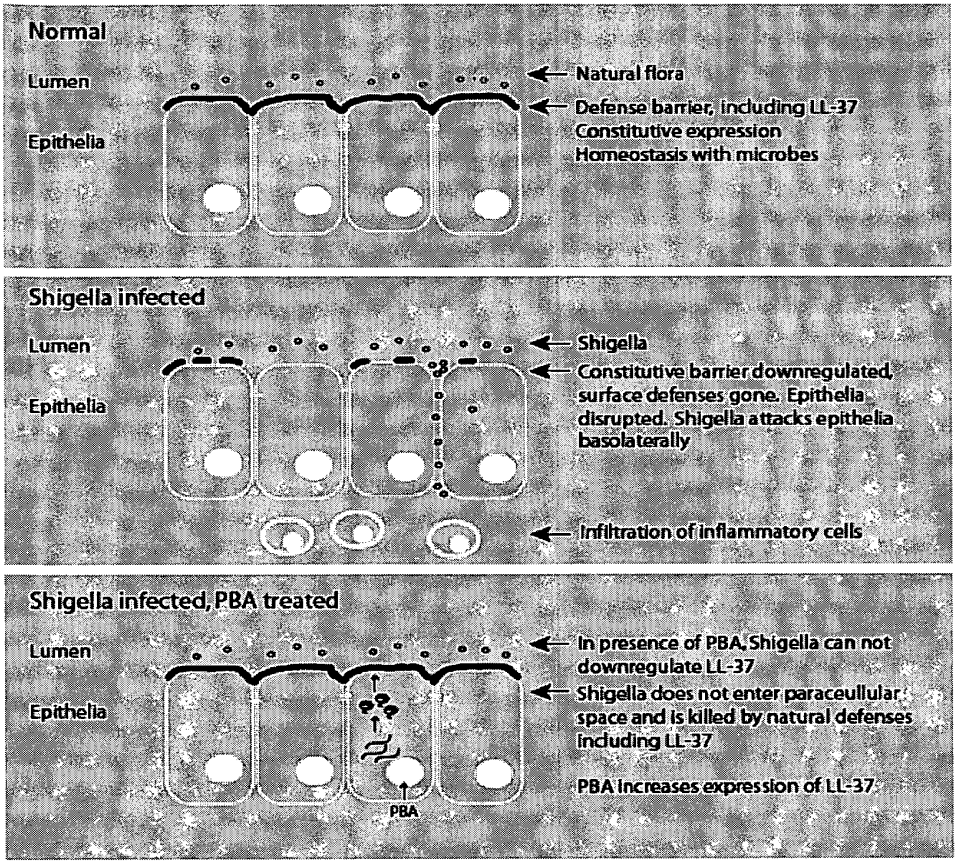


Figure 11

Figure 12


Normal
Lumen
Epithelia
Natural flora
Defense barrier, including LL-37
Constitutive expression
Homeostasis with microbes
Shigella infected
Lumen
Epithelia
Shigella
Constitutive barrier downregulated, surface defenses gone. Epithelia disrupted. Shigella attacks epithelia basolaterally
Infiltration of inflammatory cells
Shigella infected, PBA treated
Lumen
Epithelia
In presence of PBA, Shigella can not downregulate LL-37
Shigella does not enter paraceullular space and is killed by natural defenses including LL-37
PBA increases expression of LL-37
PBA

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WO 2012/028620 A1

(54) **Title**: POROUS POLYSACCHARIDE SCAFFOLD COMPRISING NANO-HYDROXYAPATITE AND USE FOR BONE FORMATION

(57) **Abstract**: The present invention relate to three dimensional porous polysaccharide matrices able to induce mineralisation of a tissue in osseous site, as well as in non- osseous site, in the absence of stem cells or growth factors.

Porous polysaccharide scaffold comprising nano-hydroxyapatite and use for bone formation

FIELD OF THE INVENTION

The present invention relates to a method for preparing a porous polysaccharide scaffold comprising hydroxyapatite, preferably nano-hydroxyapatite, that supports mineralization of tissues. The present invention further provides a porous polysaccharide scaffold obtainable by said method, and its use for bone formation.

BACKGROUND OF THE INVENTION

The topic of bone-related disorders has gained considerable attention over the past years. The use of autologous and allograft bones has been popularly implemented in clinics for overcoming bone related disorders, such as bone defect. However, the use of autologous bone is known to result in secondary trauma and allograft bone induces immune repulsion. In addition, autologous and allograft bones present serious limitations since their uses are dependent on the size and the localisation of the defect. For example, it was reported that grafts in large defects were resorbed by the body before the completion of osteogenesis, which leaves a doubt about the success of this therapy (Hoexter DL. *Bone regeneration graft materials* J Oral Implantol. 2002;28(6); Delloye C, Cornu O, Druez V, Barbier O. *Bone allografts: What they can offer and what they cannot.* J Bone Joint Surg Br. 2007 May;89(5):574-9).

To remedy to those drawbacks, many works have focus their interest into replacing natural bone by synthetically prepared implants, capable of inducing mineralisation and of supporting new bone formation. Three dimensional scaffolds have thus been explored to repair tissues that do not self develop spontaneously. Thus, scaffold-based tissues engineering has become a promising strategy in regenerative medicine, because cells alone lack the ability to form three dimensional tissues without the support of an artificial structure.

Prior art discloses porous scaffolds suitable for tissue engineering since their porous structure promotes cell colonization and tissue formation within the scaffold.

However, using said scaffolds for the treatment of bone related disorders still present various drawbacks related to the disease to be treated, as it depends on the type, size, and localisation of the damaged bone, as well as on the nature, age and sex of the subject to be treated.

Currently, many works are based on the use of bioactive and biocompatible material such as hydroxypatite. Indeed, hydroxyapatite, which is able to bond with the bone, is used as a filler to replace amputated bone or as a coating to promote bone ingrowth into prosthetic implants. However, the use of hydroxyapatite presents limitations since it is mainly effective on osseous sites.

There is currently no available technique providing bone formation which does not present any risk of rejection and which may be independent of the size and localisation of the bone to regenerate.

Consequently, there is a need for a biocompatible porous material, which can be used on any subject, independently of the type, size and localisation of the damaged bone, and is capable of promoting bone formation and providing osteoinductive properties.

SUMMARY OF THE INVENTION

The inventors have prepared porous three-dimensional polysaccharide scaffold able to provide an ideal environment for bone formation and facilitate the growth of vasculature into the material. Surprisingly and unexpectedly, the inventors have shown that polysaccharide scaffold comprising nanocristalline hydroxyapatite induce mineralisation of a tissue. Thus, by stimulating undifferentiated cells *in situ* into bone cell lineages, the invention overcomes the limitations of the prior art strategies of treatment of bone related disorders.

The inventors have thus found out very promising polysaccharide scaffolds for bone formation, in a non-osseous site, in the absence of growth factors or stem cells. The invention hence challenges the currently acknowledged techniques for treating bone related disorders and offers a wide range of possibilities disclosed hereafter.

The invention relates to a method for preparing a porous polysaccharide scaffold comprising the following step:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii),

wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably nano-hydroxyapatite.

The invention also relates to a method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent,

b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b), wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite, preferably nano-hydroxyapatite,

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

The invention further relates to a porous polysaccharide scaffold obtainable by the method of the invention.

The invention further relates to a porous polysaccharide scaffold obtainable according to the method of the invention, for use in the treatment of bone related disorders.

DETAILED DESCRIPTION OF THE INVENTION

Definition

As used herein, the term **"polysaccharide"** refers to a molecule comprising two or more monosaccharide units.

As used herein, the term **"alkaline solution"** refers to a solution having a pH strictly superior to 7.

As used herein, the term **"aqueous solution"** refers to a solution in which the solvent is water.

As used herein, the term **"porogen agent"** refers to any solid agent which has the ability to form pores within a solid structure.

As used herein, the term **"cross-linking"** refers to the linking of one polysaccharide chain to another one with covalent bonds.

As used herein, the term **"cross-linking agent"** encompasses any agent able to introduce cross-links between the chains of the polysaccharides of the invention.

As used herein, the term "**scaffold**" or **"matrix"** refers to a semi-solid system comprising a three-dimensional network of one or more species of polysaccharide chains. Depending on the properties of the polysaccharide (or mixtures of polysaccharides) used, as well as on the nature and density of the network, such structures in equilibrium can comprise various amounts of water. In the following, the terms "scaffold" and "matrix" are interchangeable.

As used herein, the term '**hydroxyapatite**', or "**micro-hydroxyapatite**" or "**HA**" refers to a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities. The OH^- ion can be replaced by fluoride, chloride or carbonate, producing fluorapatite or chlorapatite. Preferably, for the purpose of the invention, the OH^- is not replaced. Hydoxyapatite is the major component of bone and teeth matrix and gives bones and teeth their rigidity. Typically, the size of the microparticles of hydroxyapatite is comprised between 1 to 20 µm, preferably 5 and 15 µm.

As used herein, the term "**nanocristalline hydroxyapatite**", or "**nano-hydroxyapatite**", or "**n-HA**", refers to hydroxyapatite crystal particles having a size comprised between 10 and 100 nm, preferably 20 and 80 nm, preferably 30 and 70 nm, preferably between 30 and 60 nm, and most preferably about 50 nm. Preferably, the n-HA particles are needle-shaped. Preferably, the n-HA suitable for carrying out the present invention is a n-HA prepared by chemical precipitation at room temperature, for example by precipitation of a solution of phosphoric acid with a solution of calcium hydroxide.

As used herein, the term "**porous composite polysaccharide scaffold**" refers to a porous scaffold comprising polysaccharides associated with n-HA according to the invention.

As used herein, the term **"biodegradable"** refers to materials that degrade in vivo to non-toxic compounds, which can be excreted or further metabolized.

As used herein, the term **"sublimation"** refers to the physical phase transition from a solid state directly to a vapor state. More specifically, sublimation is a process in which a substance goes from a solid to a gas without going through a liquid phase. Sublimation of a solution may be obtained through the freeze-drying process.

As used herein, the term **"freeze-drying"** refers the drying of a deep-frozen material under high vacuum by freezing out the solvent (ie. water) and then evaporating it in the frozen state.

As used herein, the terms **"treating"**, "**treatment**" and **"therapy"** refer to therapeutic treatment and prophylactic, or preventative manipulations, or manipulations which stimulate bone cell differentiation or bone formation. Such expression also encompasses manipulations which postpone the development of bone disorder symptoms, and/or reduce the severity of bone disorders and/or such symptoms that will or are expected to develop from a bone disorder. The terms further include ameliorating existing bone disorder symptoms, preventing additional symptoms, or preventing or promoting bone growth.

As used herein, the expression "**bone tissue**" refers to calcified tissues (e.g., calvariae, tibiae, femurs, vertebrae, teeth), bone trabeculae, the bone marrow cavity, the cortical bone, which covers the outer peripheries of the bone trabeculae and the bone marrow cavity, and the like. The expression "bone tissue" also encompasses bone cells that are generally located within a matrix of mineralized collagen; blood vessels that provide nutrition for the bone cells; bone marrow aspirates: joint fluids: bone cells that are derived from bone tissues; and may include fatty bone marrow. Finally, bone tissue includes bone products such as whole bones, sections of whole bone, bone chips, bone powder, bone tissue biopsy, collagen preparations, or mixtures thereof. For the purposes of the present invention, the term "bone tissue" is used to encompass all of the aforementioned bone tissues and products, whether human or animal, unless stated otherwise.

As used herein, the expression **"bone-related disorders**" includes disorders of bone formation and bone resorption. Preferably, the expression "bone related disorders" refers to diseases associated with insufficiency of bone formation or bone loss.

Non-limiting examples of bone related disorders are rickets, osteoporosis osteomalacia, osteopenia, bone cancer, arthritis, rickets, bone fracture, bone defects, osteolytic bone disease, osteomalacia, bone frailty, loss of bone mineral density achondroplasia, cleidocranial dysostosis, Paget's disease, osteogenesis imperfecta, osteopetrosis, sclerotic lesions, pseudoarthrosis, periodontal disease, anti-epileptic drug induced bone loss, weightlessness induced bone loss, postmenopausal bone loss, osteoarthritis, infiltrative disorders of bone, metabolic bone diseases, organ transplant related bone loss, adolescent idiopathic scoliosis, glucocorticoid-induced bone loss, heparin-induced bone loss, bone marrow disorders, malnutrition, calcium deficiency, rheumatoid arthritis, hypogonadism, HIV associated bone loss, tumor-induced bone loss, cancer-related bone loss, hormone ablative bone loss, multiple myeloma drug- induced bone loss, facial bone loss associated with aging, cranial bone loss associated with aging, jaw bone loss associated with aging, skull bone loss associated with aging, and bone loss associated with space travel.

Preferably, the bone related disorders, as used herein, are bone fracture, large bone defects, rickets, osteoporosis, osteogenesis imperfecta, osteomalacia, osteopenia, bone cancer, osteolytic bone disease, bone frailty and/or loss of bone mineral density.

Porous polysaccharide scaffolds and methods for preparing thereof

In a first object, the invention relates to a method for preparing a porous polysaccharide scaffold comprising the following step:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii), wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably nano-hydroxyapatite.

The concentration of the porogen agent affects both the total porosity and the size of the pores formed in the scaffolds, so that the porosity and the pore size can be under the control of the concentration of said porogen agent.

Non-limiting examples of porogen agents are sodium chloride, calcium chloride, ammonium carbonate, ammonium bicarbonate, calcium carbonate, sodium carbonate, and sodium bicarbonate and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich (St. Louis, Michigan, US).

Preferably, in the context of the present invention, the porogen agent is chosen from sodium chloride, calcium chloride or mixtures thereof.

Alternatively, the porogen agent may be an inorganic salt that can be dissolved once the cross-linked polysaccharide scaffold is immersed in water. An example of such a porogen agent includes saturated salt solution, which would be dissolved progressively.

Typically, the weight ratio of the polysaccharide to the porogen agent is in a range1:50 to 50:1, preferably from 1:30 to 30:1, preferably from 1:12 to 12:1.. In a preferred embodiment, said weight ratio of the polysaccharide to the porogen agent is about 12:14.

Typically, the aqueous solution of step iii) is water.

Alternatively, the aqueous solution of step iii) is a buffer solution. Non-limiting examples of buffer solution are PBS (Phosphate buffered saline), EDTA (ethylenediaminetetraacetic acid), TAPS (3-{ [tris(hydroxymethyl)methyl]amino} propanesulfonic acid), Bicine (N,N-bis(2-hydroxyethyl)glycine), Tris (tris(hydroxymethyl)methylamine), Tricine (N-tris(hydroxymethyl)methylglycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid), Cacodylate (dimethylarsinic acid), SSC (saline sodium citrate), MES (2-(N-morpholino)ethanesulfonic acid) and mixtures thereof.

Alternatively, the aqueous solution of step iii) is an acidic solution. The acid may be selected from the group consisting of citric acid, hydrochloric acid, acetic acid, formic acid, tartaric acid, salicylic acid, benzoic acid, and glutamic acid.

Preferably, the aqueous solution of step iii) is a buffer solution. Most preferably, the aqueous solution of step iii) is phosphate buffer saline (PBS).

Preferably, the solvent of step ii) is an inorganic solvent.

In one embodiment, the method of the invention may comprise a further step, consisting of freeze-drying the scaffold obtained at step iv). Freeze-drying may be performed with any apparatus known in the art. There are essentially three categories of freeze dryers: rotary evaporators, manifold freeze dryers, and tray freeze dryers. Such apparatus are well known in the art and are commercially available such as a freeze-dryer Lyovac (GT2, STERIS Rotary vane pump, BOC EDWARDS). Basically, the vacuum of the chamber is from 0.1 mBar to about 6.5 mBar. The freeze-drying is performed for a sufficient time sufficient to remove at least 98.5 % of the water, preferably at least 99% of the water, more preferably at least 99.5%.

In another embodiment, the method of the invention may comprise a further step consisting of hydrating the scaffold as prepared according to the invention. Said hydration may be performed by submerging the scaffold in an aqueous solution (e.g., de-ionized water, water filtered via reverse osmosis, a saline solution, or an aqueous solution containing a suitable active ingredient) for an amount of time sufficient to produce a scaffold having the desired water content. Typically, when a scaffold comprising the maximum water content is desired, the scaffold is submerged in the aqueous solution for an amount of time sufficient to allow the scaffold to swell to its maximum size or volume. Typically, the scaffold is submerged in the aqueous solution for at least about 1 hour, preferably at least about 2 hours, and more preferably about 4 hours to about 24 hours. It is understood that the amount of time necessary to hydrate the scaffold to the desired level will depend upon several factors, such as the composition of the used polysaccharides, the size (e.g., thickness) of the scaffold, and the temperature of the aqueous solution, as well as other factors.

Preferably, the hydrated scaffold comprises more than 80% of water, preferably 90% of water, most preferably 95 % of water.

In a second aspect, the invention relates to a method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, and one cross-linking agent,

b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b), wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite, preferably nano-hydroxyapatite,

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

It is an essential feature of the invention that step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a). Typically, temperature and time are the main factors to control the cross-linking of the aqueous solution. To avoid or to seriously limit the cross-linking of the polysaccharide, the aqueous solution may be prepared at a temperature under 37°C, more preferably comprised between 4°C and 25°C. Moreover, the step b) may be performed as quickly as possible to avoid the cross-linking of said polysaccharide.

Once the aqueous solution is prepared, it is frozen. The freezing of the aqueous solution may be performed at different rates (e.g., °C/min). Typically, the freezing may be performed at rate from about 1°C/min to about 200°C/min, preferably from about 1°C/min to about 20°C/min, and most preferably from about 5°C/min to about 10°C/min. The solution may be frozen in liquid nitrogen or in dried ice.

When the aqueous solution is frozen, sublimation may take place. In a preferred embodiment, the method for preparing porous polysaccharide scaffolds according to the present invention includes a freeze-drying process. Therefore, according to the invention, the freeze-drying process has to take place before the cross-linking process occurs in the aqueous solution. Freeze-drying may be performed with any apparatus known in the art. There are essentially three categories of freeze dryers: rotary evaporators, manifold freeze dryers, and tray freeze dryers. Such apparatus are well known in the art and are commercially available such as a freeze-dryer Lyovac (GT2, STERIS Rotary vane pump, BOC EDWARDS). Basically, the deep-frozen aqueous solution is placed in a chamber. Then the chamber temperature is increased to a level higher than the boiling point of the

liquefied vapour, whereby the vapour is vaporized and removed. Typically, the temperature of chamber may be from -70 °C to -1°C, preferably from -70°C to -40°C, further preferably about -50°C to -40°C. The heating of the chamber is accompanied with a vacuum flow to decrease the pressure of the chamber. Typically, the vacuum of the chamber is from 0.1 mBar to about 6.5 mBar. Typically, the freeze-drying is performed for a sufficient time sufficient to remove at least 98.5 % of the water, preferably at least 99% of the water, more preferably at least 99.5%.

The freezing of the aqueous solution causes the formation of ice particles from the water. Without to be bound by any theory, under the temperature and pressure condition described above, water included in the frozen solution is sublimed, and thus, thereby leaving interstices in the material in the spaces previously occupied by the ice particles, and accordingly porous polysaccharide scaffolds are produced. Surprisingly, the cross-linking process occurs during the freeze-drying process.

The material density and pore size of the resultant scaffold may be therefore varied by controlling the rate of freeze-drying of the frozen aqueous solution. The essential parameter in a freeze-drying process is the vacuum rate.

For the purpose of the present invention, any type of polysaccharide can be used. Synthetic or natural polysaccharide may be alternatively used in the context of the invention. Non-limiting examples of suitable polysaccharide for implementing the present invention are dextran, agar, alginic acid, hyaluronic acid, inulin, pullulan, heparin, fucoidan, chitosan, scleroglucan, curdlan, starch, cellulose and mixtures thereof. Chemically modified polysaccharides bearing for instance acidic groups (carboxylate, sulphate, phosphate), amino groups (ethylene amine, diethylaminoethylamine, propylamine), hydrophobic groups (alkyl, benzyl) can be included. Saccharide structures and oligosaccharides that may be used to produce the desired materials include but are not limited to ribose, glucose, mannose, galactose, fructose, sorbose, sorbitol, mannitol, iditol, dulcitol and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich (St. Louis, Michigan, US).

Typically, the average molecular weight of the polysaccharides is from about 5,000 Daltons to about 2,000,000 Daltons, preferably from about 100,000 Daltons to

about 500,000 Daltons. Typically, the polysaccharide used to prepare the scaffold of the invention is a neutral polysaccharide such as dextran, agar, pullulan, inulin, scleroglucan, curdlan, starch, cellulose and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a positively charged polysaccharide such as chitosan, DEAE-dextran, DEAE-pullulan, EA-pullulan and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a negatively charged polysaccharide such as alginic acid, hyaluronic acid, heparin, fucoidan and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a mixture of neutral and negatively charged polysaccharides. Typically, the negatively charged polysaccharides represent 1 to 20%, preferably 5 to 10% of the mixture. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a mixture of neutral and positively charged polysaccharides. Typically, the positively charged polysaccharides represent 1 to 20%, preferably 5 to 10% of the mixture.

Preferably, for the purpose of the invention, said polysaccharide is selected in the group consisting of dextran, pullulan, agar, alginic acid, starch, hyaluronic acid, inulin, heparin, fucoidan, chitosan and mixtures thereof. In one particular embodiment of the invention, said polysaccharide is a mixture of pullulan and dextran. Typically, the weight ratio of pullulan/dextran is in a range from 95:5 to 95:5 (w/w), preferably in a ration of 75:25 (w/w). In another embodiment of the invention, said polysaccharide is a mixture of pullulan, dextran and fucoidan. Typically, the weight ratio of pullulan/dextran/fucoidan is in a range from about 70:20:10 to about 50:20:30, preferably from about 70:20:10 to about 50:30:20, and most preferably in a ratio of about 73:22:5 (w/w). The presence of fucoidan in the porous polysaccharide scaffold of the invention is highly advantageous since fucoidan promotes vascularisation.

Typically, the covalent cross-linking agent is selected from the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride ($POCl_3$), epichlorohydrin, formaldehydes, carbodiimides, glutaraldehydes, any other compound that is suitable for crosslinking a polysaccharide and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich (St. Louis, Michigan, US). Preferably, for the purpose of the present invention, said cross-linking agent is STMP. Typically, the concentration of the

covalent cross-linking agent in the aqueous solution (w/v) is from about 1% to about 6%, more preferably from about 2% to about 6%, most preferably from about 2% to about 3%. Typically, the weight ratio of the polysaccharide to the cross-linking agent is in a range from 20:1 to 1:1, preferably from 10:1 to 2:1.

In the context of the present invention, nano-hydroxyapatite may be a commercial nano-hydroxyapatite, such as those commercialised by Inframat Corporation or Fluidinova. Preferably, nanocristalline hydroxyapatite useful in the context of the present invention is obtained through chemical precipitation at room temperature of a solution of phosphoric acid, at a concentration comprised between 0.3 to 1M, preferably 0.6M, with a solution of calcium hydroxide, at a concentration comprised between 0.5 to 1.5M, preferably 1M. Typically, the concentration of hydroxyapatite in the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v). Typically, the concentration of nano-hydroxyapatite in the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v).

In one embodiment, the alkaline aqueous solution of step a) or step i) comprising hydroxyapatite, preferably nano-hydroxyapatite, may be poured in a mould before step b) or step ii), so that the porous polysaccharide scaffold obtained with the method of the invention can take a desired form. Any geometrical moulds may be used according to the invention. Different sizes may also be envisaged. The mould may be made of any material, but preferred material includes non sticky surfaces such as Teflon.

Alternatively, the scaffolds of the invention may be cut and shaped to take a desired size and form.

The methods of the invention can further include the step of sterilizing the scaffold using any suitable process. The scaffold can be sterilized at any suitable point, but preferably is sterilized before the scaffold is hydrated. A suitable irradiative sterilization technique is for example an irradiation with Cesium 137, 35 Gray for 10 minutes. Suitable non-irradiative sterilization techniques include, but are not limited to, UV-exposure, gas plasma or ethylene oxide methods known in the art. For example, the scaffold can be sterilized using a sterilisation system which is

available from Abtox, Inc of Mundelein, Illinois under the trade mark PlazLyte, or in accordance with the gas plasma sterilization processes disclosed in US-5413760 and US-5603895.

The scaffold produced by the methods of the invention can be packaged in any suitable packaging material. Desirably, the packaging material maintains the sterility of the scaffold until the packaging material is breached.

In a further embodiment, the alkaline solution of step i) or a) further comprises a drug. The invention thus provides porous polysaccharide scaffold comprising a drug. Typically, said drug is a drug having an acknowledged therapeutic effect, such as hormones radioactive substance, fluorescent substance, chemotactic agent, antibiotic, steroidal or non-steroidal analgesic, immunosuppressant, or anti-cancer drug, drugs belonging to the pharmaceutical class of statins. Preferably, said drug belongs to the pharmaceutical class of statins. As used herein, "statins" refers to a pharmaceutical class of HMG-CoA reductase inhibitors. It has been recently shown that some of the drugs from this pharmaceutical class play a role in the process of bone formation. Preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin pitavastatin, rosuvastatin, pravastatin, fluvastatin and simvastatin. More preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin and simvastatin. Said statins are highly appropriate in the context of the present invention since they play a role in the bone formation.

In a further embodiment, the alkaline solution further comprises a bioactive substance. Typically, said bioactive substance is a substance known for playing an important role in various mechanisms such as modification of cellular pathways and modification of cellular or tissular responses. Said bioactive substance is chosen among growth factors, cytokines (lymphokines, interleukins, and chemokines), antioxidant molecules, angiogenic molecule, anti-angiogenic agents, immunomodulating agents, proinflammatory cytokines, antiinflammatory cytokines, plasma-derived bioactive substances, PRP (platelet rich plasma)-derived substances, soluble adhesion molecules.

In a third aspect, the invention relates to porous polysaccharide scaffolds obtainable by the methods of the invention. These porous polysaccharide scaffolds are indeed the only ones which have the remarkable properties provided by the

invention. When the method of preparing the porous polysaccharide scaffold according to the invention involves the use of a porogen agent, the concentration of the porogen agent affects the size of the pores formed in the scaffolds. Therefore, in this particular embodiment, the size of the pores can be under the control of the concentration of said porogen agent. Typically, the average pore size of the scaffold is from about 1µm to about 500 µm, preferably from about 10 µm to about 200 µm. Typically, the density of the pores (or porosity) is from about 4% to about 75%, preferably from about 4% to about 50%. The person skilled in the art may provide desired properties to the porous polysaccharide scaffold according to the invention. Typically, the person skilled in the art may add one or more compounds chosen in the group consisting of a biomolecule, a bioactive agent, a drug, an anti-inflammatory agent, an additive, an antimicrobial agent, a colorant, a surfactant and a differentiation agent. The techniques for incorporating said compounds in the porous polysaccharide scaffold of the invention completely falls within the ability of the person skilled in the art. Typically, said compounds may be added directly the alkaline solution of step i) or a) of the method of the invention. In this particular embodiment, the compound would be within the structure of the porous polysaccharide scaffold of the invention. Alternatively, said compounds can be incorporated into the porous polysaccharide scaffold during a step consisting of hydrating said scaffold with a solution of the compound.

In one embodiment, the porous polysaccharide scaffold of the invention further comprises one or more biomolecules. Non-limiting examples of biomolecules are drugs, hormones, radioactive substances, fluorescent substances, chemicals or agents, chemotactic agents, antibiotics, steroidal or non-steroidal analgesics, immunosuppressants, anti-cancer drugs, short chain peptides, glycoprotein, lipoprotein, cell attachment mediators, biologically active ligands, integrin binding sequence, ligands, small molecules that affect the up-regulation of specific growth factors, tenascin-C, hyaluronic acid, chondroitin sulphate, fibronectin, decorin, thromboelastin, thrombin-derived peptides, and mixtures thereof. The presence of said biomolecules in the porous polysaccharide scaffold of the invention may enhance treatment effects, enhance visualization, indicate proper orientation, resist infection, promote healing, may increase softness or any other desirable effects. In another embodiment, the porous polysaccharide scaffold of the invention further

comprises a bioactive substance. Typically, said bioactive substance is a substance known for playing an important role in various mechanisms such as modification of cellular pathways and modification of cellular or tissular responses. Said bioactive substance is chosen among growth factors, cytokines (lymphokines, interleukins, and chemokines), antioxidant molecules, angiogenic molecule, anti-angiogenic agents, immunomodulating agents, proinflammatory cytokines, antiinflammatory cytokines, plasma-derived bioactive substances, PRP (platelet rich plasma)-derived substances, and soluble adhesion molecules.

In a further embodiment, the porous polysaccharide scaffold of the invention further comprises one or more drug. Typically, said drug is a drug having an acknowledged therapeutic effect, such as hormones radioactive substance, fluorescent substance, chemotactic agent, antibiotic, steroidal or non-steroidal analgesic, immunosuppressant, or anti-cancer drug, drugs belonging to the pharmaceutical class of statins. Preferably, said drug belongs to the pharmaceutical class of statins. Preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin pitavastatin, rosuvastatin, pravastatin, fluvastatin and simvastatin. More preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin and simvastatin. Said statins are highly appropriate in the context of the present invention since they play a role in the bone formation

In another embodiment, the porous polysaccharide scaffold of the invention further comprises anti-inflammatory agents. Non-limiting examples of anti-inflammatory agents are indomethacin, salicylic acid acetate, ibuprofen, sulindac, piroxicam, and naproxen; thrombogenic agents, such as thrombin, fibrinogen, homocysteine, and estramustine; and radio-opaque compounds, such as barium sulfate, gold particles and iron oxide nanoparticles (USPIOs) and mixtures thereof.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises additives. The amount of the additive used depends on the particular application of the porous polysaccharide scaffold of the invention and may be readily determined by the person skilled in the art using routine experimentation.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises an antimicrobial agent. Suitable antimicrobial agents are

well known in the art. Non-limiting examples of suitable antimicrobial agents are alkyl parabens, such as methylparaben, ethylparaben, propylparaben, and butylparaben; cresol; chlorocresol; hydroquinone; sodium benzoate; potassium benzoate; triclosan and chlorhexidine and mixture thereof. Other examples of antibacterial agents and of anti-infectious agents that may be used are, in a non-limiting manner, rifampicin, minocycline, chlorhexidine, silver ion agents and silver-based compositions and mixtures thereof.

In a further embodiment, the porous polysaccharide scaffold of the invention further comprises at least one colorant to enhance the visibility of the scaffold. Suitable colorants include dyes, pigments, and natural coloring agents. Non-limiting examples of suitable colorants are alcian blue, fluorescein isothiocyanate (FITC) and FITC dextran and mixtures thereof.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises at least one surfactant. Surfactant, as used herein, refers to a compound that lowers the surface tension of water. The surfactant may be an ionic surfactant, such as sodium lauryl sulfate, or a neutral surfactant, such as polyoxyethylene ethers, polyoxyethylene esters, and polyoxyethylene sorbitan and mixtures thereof.

In one embodiment, the porous polysaccharide scaffold of the invention further comprises a differentiation agent. Preferably, such a differentiation agent is an agent involved in bone formation. Alternatively, such a differentiation agent is an agent involved in osteogenesis, angiogenesis or wound healing. Preferably, such a differentiation agent is a growth factor. Non-limiting examples of growth factor suitable for the purpose of the present invention are epidermal growth factor (EGF), insulin-like growth factor (IGF-I, IGF-II), transforming growth factor beta (TGFβ), heparin binding growth factor (HBGF), stromal derived factor (SDF-1), vascular endothelial growth factors (VEGF), fibroblast growth factors (FGFs), platelet derived growth factors (PDGF), parathyroid hormone (PTH), parathyroid hormone related peptide (PTHrP), basic fibroblast growth factor (bFGF); TGFβ superfamily factors; bone morphogenetic proteins (BMPs) preferably BMP2, BMP3, BMP4, BMP5, BMP7, somatropin, growth differentiation factor (GDF) and mixtures thereof.

Typically, the growth factor is present at a concentration comprised from 1 ng to 100 μg per porous polysaccharide scaffold of the invention.

In another embodiment, the porous polysaccharide scaffold of the invention further comprises cells, such as yeast cells, mammalian cells, insect cells, and plant cells.

Preferably, said cell is a mammalian cell. Non-limiting examples of mammalian cells suitable for the purpose of the invention are differentiated cells such as chondrocytes, fibrochondrocytes, osteocytes, osteoblasts, osteoclasts, synoviocytes, epithelial cells and hepatocytes or stem cells, embryonic stem cells, induced progenitor stem cells (iPS), mesenchymal stem cells from different sources, bone marrow, adipose tissue, peripheral blood progenitor cells, cord blood progenitor cells, genetically transformed cells and mixtures thereof. Most preferably, the mammalian cells comprised in the porous polysaccharide scaffold according to the invention are adipose derived stroma cells. Typically, the mammalian cells comprised in the porous polysaccharide scaffold are present at a cell density comprised between 200 cells/mm^3 to 35 000 cells/mm^3.

In a fourth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for bone generation.

As used herein, the expression “bone generation” encompasses “bone repair” and “bone development”.

In a fifth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for stimulating ectopic mineralized tissue formation. In the context of the present invention, the expression "ectopic" refers to a non osseous tissue. Therefore, the invention also relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for inducing mineralized tissue in a non-osseous site.

Preferably, said stimulation of ectopic mineralization occurs in absence of stem cells and/or growth factors. Indeed, the inventors have shown that the porous polysaccharide scaffold according to the invention has the ability to induce mineralized tissue in a non-osseous site and in an osseous site (calvaria site or femoral condyle), even in the absence of stem cells and/or growth factors. Therefore, the invention provides a porous polysaccharide scaffold useful for stimulating mineralized tissue formation in osseous site, as well as in non-osseous site, in the presence as well as in the absence of stem cells and/or growth factors.

Use of the porous polysaccharide scaffold according to the invention

The inventors have shown that implanting porous polysaccharide scaffold according to the invention lead to the stimulation of a dense collagen network and blood vessel formation as well as the recruitment of osteoblast-like cells. Said implantation of scaffolds according to the invention in subcutaneous site leads to the formation of a dense mineralized tissue, and thus to bone formation.

The inventors have shown that the scaffold of the invention, when implanted, retains growth factor such as VEGF and BMP. The inventors also evidenced that the ability of retaining said growth factor was higher for the scaffold comprising n-HA, compared to a scaffold not comprising n-HA.

In a sixth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use in the treatment of bone related disorders. The inventors have indeed shown the ability of the porous polysaccharide scaffold according to the invention to stimulate the production of an extracellular mineralized matrix, probably through differentiation of cells into bone cells. Thus, the inventors evidenced that the scaffold of the invention is useful for the treatment of bone related disorders.

In a seventh aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use as a polysaccharide scaffold.

Typically, the size and the shape of the porous polysaccharide scaffold can be adapted to the type and size of the bone to replace, and to the localization of said bone. Preferably, the shape of the scaffold is a sphere, a cylinder, a cube or a rectangular cuboid. Preferably, the size of said scaffold is comprised between 0.5 mm and 30 cm. Typically, the polysaccharide scaffold of the invention may be is implanted as follows: the lyophilized scaffold is placed within the defect and its size is adapted to the size of defect. For example, for the implantation in calvaria site in mouse, defects of 4 mm of diameter and 500 µm of depth were performed and the matrices were apposed onto the host tissue. In mice, bone defect performed in the femoral condyle is around $1mm^3$. In rat, the critical size defect performed in the

femoral condyle is 5 mm of diameter and 3 mm of depth. These bone defects are filled with the matrices. For segmental bone defect in large animal (sheep or goat), a resection of 2.5 cm is performed at metatarsus and cylinder of polysaccharide scaffold is placed within the defect. Analysis of the newly formed tissue within the defect is performed between 15 days to 12 months. The person skilled in the art is award of the routine suitable techniques for analyzing said newly formed tissue. Typically, said analysis may be performed using several invasive methods such as histomorphometry as gold standard technique. Alternatively, said analysis may be performed using non invasive imaging approaches such as Magnetic Resonance Imaging (MRI), X Ray micro Computed Tomography (micro-CT), Single Photon Emission Computarized Tomography (SPECT) or radiological analysis. The choice of the suitable technique is dependent on the type of bone in small and large animals, or humans.

FIGURES LEGENDS

Figure 1: Porous polysaccharide scaffold.

Macroscopic view of hybrid porous discs with n-HA before (Figure 1A) and after (Figure 1B) rehydration with phosphate buffer saline (PBS). The scale bar corresponds to 1 mm.

Figure 2: Electron Microscopy of a freeze-dried polysaccharide scaffold.

The morphology of freeze-dried scaffolds was analyzed by scanning electron microscopy (Figure 2A). After rehydration in PBS, porosity of hydrated scaffolds was observed with Environnemental Scanning Electron Microscopy (ESEM Philips XL 30) (Figure 2B).

Figure 3: Healing of critical size defects in nude mice by the polysaccharide-based matrices.

Micro-CT images of calvaria defects filled with polysaccharide matrices without n-HA (Figure 3A), or with the polysaccharide scaffold (Figure 3B), loaded (on left side) or not (on right side) with $5x10^5$ differentiated adipose derived stromal cells (ADSCs). Imaging on the same animal for each type of scaffold was performed after 15, 30, 60 and 84 days of implantation, and resulting images are respectively referred to as D15, D30, D60, D84. Quantitative analysis of the Tissue Mineral

Density (TMD) of implanted polysaccharide scaffold. Calvaria bone was used as a control (Figure 3C).

Figure 4: Ectopic mineralized tissue formation in subcutaneous site induced by the polysaccharide scaffold.

(A) Micro-CT images at Days 15, 30 and 60 of a mouse implanted with two discs of the polysaccharide scaffold (n-HA/scaffold) (left site) and one disc previously seeded with $5x10^5$ differentiated ADSCs (right site).

(B) Macroscopic view at D60.

(C) Quantitative analysis of the tissue mineral density (TMD).

(D) Histological examination of undecalcified (D1; magnification x10) (stained by Goldner's trichrome) and decalcified (D2; magnification x2) (D3; magnification x20) sections (Masson's staining) obtained at Day 60.

(E) Von Kossa staining performed on explanted materials at Day 30 and Day 60. Control was performed using the paraffin-embedded composite matrix before implantation (magnification x2).

Figure 5 : Matrix+n-HA (MATRI+) induces mineralization in ectopic site of mice.

(A) Representative micro-CT images of the subcutaneous implantation of the Matrix alone on the left side (indicated by an arrowed doted line) and Matrix+n-HA (MATRI+) on right side (indicated by an arrowed plain line), after 15 (D15), 30 (D30) and 60 days (D60) of implantation in Balb/c mice.

(B) Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from reconstructed three-dimensional micro-CT images with Microview Image analyser of the Matrix (white rectangle) and Matrix+n-HA (MATRI+) (black rectangle). Data are presented as means ± standard deviation for n=8. The symbol ** indicates a statistically significant difference compared to the other groups <0.01.

Figure 6 : Matrix+n-HA induces formation of a collagen-based mineralized tissue: histological analyis of the newly formed tissue.

(A) Representative histological undecalcified sections of the Matrix and Matrix+n-HA (MATRI+) samples implanted subcutaneously in mice, after 15 days (D15) and 60 days (D60) : Von Kossa staining.

(B) Representative histological decalcified sections of Matrix+n-HA (MATRI+) 60 days after implantation : Goldner staining, The images showed a high

dense collagen tissue around the implant that colonizes the scaffold, with osteoblast-like cells as indicated by the white arrows, and numerous vessels inside the collagen tissue indicated by the black arrows.

Figure 7 : XRD patterns of matrices before surgery (D0) and 15 days (D15) after subcutaneously implantation in mice.

(A) Matrix+n-HA (MATRI+) ; (B) Matrix without n-HA

Specific peaks of hydroxyapatite (HA) are only observed in the XRD patterns after 15 days of implantation of MATRI+. Peaks of Halite (H) due to sample processing, are observed in all spectra. The XRD patterns obtained at day 30 and day 60 are similar than those observed at D15 for both groups (data not shown).

Figure 8 : Matrix+n-HA (MATRI+) retained endogeneous osteoinductive and angiogenic factors.

Measurement by ELISA of BMP2 (A) and VEGF165 (B), retained in the tissue formed within the Matrix (white rectangle) and Matrix+n-HA (MATRI+) (black rectangle) when implanted subcutaneously at D15, D30 and D60. Results are expressed in pg of growth factors retained per µg of proteins quantified by BCA. Data are presented as means ± standard deviation for n=6 samples. The symbols * and ** indicate a statistically significant difference compared to the other groups with $p<0.05$ and <0.01, respectively.

Figure 9 : Matrix+n-HA (MATRI+) induces a high mineralization of tissue in a critical size bone defect performed in the femoral condyle of rats.

(A) Representative micro-CT images of the femoral condyle of rats, 15 days (D15), 30 days (D30) and 90 days (D90) after implantation without scaffold (empty), with Matrix or Matrix+n-HA (MATRI+).

(B) Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from reconstructed three-dimensional micro-CT images of the empty group (white rectangle), the Matrix group (grey rectangle) and Matrix+n-HA (MATRI+) (black rectangle). Data are presented as means ± standard deviation for n=4. The symbol ** indicates a statistically significant difference compared to the other groups with $p<0.01$.

Figure 10 : Matrix+nHA (MATRI+) induces a high mineralized bone tissue in a critical size bone defect performed in the femoral condyle of rats after 90 days of implantation; histological analysis of the newly formed tissue.

(A) Representative histological undecalcified sections of Empty, Matrix and Matrix+n-HA (MATRI+) samples implanted in the femoral condyle of rats, after 90 days of implantation: Von Kossa staining. The arrows indicated the position of the bone defect.

(B) Representative histological decalcified sections of of Empty, Matrix and Matrix+nHA samples 90 days after implantation : Goldner staining, A fibrous tissue was formed in the empty bone defect, while bone formation occurred in direct contact of the matrix and was enhanced within the MATRIX+ implant.

EXAMPLE

Example 1 : Implantation of the scaffold of the invention in calvaria site of athymic mice.

Materials and Methods

Nano-hydroxyapatite preparation

Nano-hydroxyapatite (n-HA) was prepared by wet chemical precipitation using a 0.6M solution of Phosphoric acid (H_3PO_4 Rectapur, Prolabo®, France) and a 1M solution of calcium hydroxide ($CaOH_2$ Alfa Aesar, Germany). 100 ml of H_3PO_4 solution were added dropwise in 100 ml of $CaOH_2$ solution during 30 minutes under vigorous stirring at room temperature. At the end of reaction, pH was adjusted to 9 using $0.4.10^{-3}$ mol of a 0.6 M sodium hydroxide solution, then stirring was continued during 12 hours.

Nano-hydroxyapatite (n-HA) has been characterized by transmission electron microscopy (TEM), scanning electron microscopy and by FTIR analysis. TEM revealed n-HA needle-shaped crystals of 50 nm long. FTIR analysis showed specific bands of phosphate ions of at 559 cm^{-1}, 601 cm^{-1} and 1018 cm^{-1} and a non-specific carbonate band 1415 cm^{-1}.

Preparation of composite polysaccharide scaffolds (MATRI+)

Macroporous composite scaffolds (MATRI+) were prepared using a blend of pullulan/dextran 75:25 (pullulan, MW 200,000, Hayashibara Inc, Dextran MW 500,000, Pharmacia), prepared by dissolving 9 g of pullulan and 3 g of dextran into 27 mL of distilled water containing 14g of NaCl and 13 mL of nano-hydroxyapatite suspension (n-HA, 6.36% w/v). Chemical cross-linking was carried out using trisodium trimetaphosphate STMP (Sigma) under alkaline condition. Briefly, 1 mL of 10M sodium hydroxide was added to 10 g of the polysaccharide blend, followed by the addition of 1 mL of water containing 300 mg of STMP. After incubation at 50°C for 15 min, resulting scaffolds were cut into 6mm diameter discs, neutralized in PBS 10X (pH 7.4) then washed extensively with a 0.025% NaCl solution. After a freeze-drying step, porous composite polysaccharide scaffolds were stored at room temperature until use. Fluorescent scaffolds were prepared by adding 1% of

Fluorescein IsoThioCyanate (FITC) dextran (Sigma, St. Louis MO, USA) to the mixture before cross-linking.

ADSC cultures and osteogenic differentiation

Adipose Derived Stromal Cells (ADSCs) were isolated from human adipose tissue after a digestion with 0.1% (w/v) collagenase type I and cultured as previously described by Gimble et al, 2007. The remaining Stromal Vascular Fraction (SVF) was cultured in a basal medium (DMEM F12 medium (Invitrogen) supplemented with 10% (v/v) Foetal Bovine Serum (FBS) or in an osteogenic medium for inducing osteoblastic differentiation of ADSCs (IMDM medium (Invitrogen), supplemented with 10% (v/v) FBS (Lonza), 10^{-8} M dexamethasone (Sigma), 50 mg/ml ascorbic acid (Sigma) and 10 mM β-glycerophosphate (Sigma)).

Experimental models in nude mice

Orthotopic new bone formation was assessed on calvaria site of athymic mice. Twelve weeks-old nude mice were anesthetized with an isoflurane/N2O mixture and were subjected to surgery to make a 4 mm diameter full thickness on the left and right parietal bone using a trephine dental burr. Disk-shaped matrices without n-HA (Group 1) and composite polysaccharide scaffold MATRI+ containing n-HA (Group 2) were implanted on top of the periosteum of the parietal bone. Group 3 corresponds to mice implanted with the composite polysaccharide scaffold associated with differentiated ADSCs one week before implantation.

To study ectopic bone formation, polysaccharide-based matrices (Group 1), composite polysaccharidescaffold without cells (Group 2), or matrices previously seeded with differentiated ADSCs (Group 3), were implanted into dorsal, subcutaneous spaces of athymic mice (female, 12 weeks old). Four scaffolds were implanted by mice. Bone formation was followed by a non invasive high resolution X-ray tomography (micro-CT) analysis performed 15, 30 and 60 days after implantation and by histological examination at the end of the experiment (D60).

High resolution X-ray tomography (micro-CT) analysis

Mice were scanned in an *in vivo* Explore Locus SP X-Ray micro-computerized tomography (micro-CT) device (General Electric) at an isotropic

resolution of 45 µm. Reconstruction of the parietal and subcutaneous region was performed following correction of rotation centre and calibration of mineral density. Bone analysis was performed using the "Advanced Bone Analysis"™ software (GE). Thresholding of grey values was performed using the histogram tool in order to separate mineralized elements from background. The density of mineralized tissue (TMD) was determined in the region of interest (ROI).

Histological evaluation

At the end of the experimental periods, mice were euthanized and samples were dissected out and fixed in 3.7% (v/v) paraformaldehyde in PBS 0.1M pH 7.4. One part of the samples were decalcified and embedded in paraffin. Permanent sections of 7 micron were stained with hematoxylin and eosin and Masson trichrome dye. The other part of the samples were embedded in methylmethacrylate as described by Schenk et al, 1984. Longitudinal sections (15 µm thick) were prepared using a Leica microtome and tungsten carbide blades. Sections were stained with Goldner's trichrome, Von Kossa, and observed using a Nikon Eclipse 80i microscope. Pictures were generated using a DXM 1200 C (Nikon) CCD camera.

Results

3D porous matrices (Figure 1) were obtained according to the methods disclosed in the PCT patent applications WO2009/047346 and WO2009/047347, with n-HA included in the starting formulation. n-HA in suspension (6.36% (w/v)) allowed an homogeneous dispersion of the HA nanoparticles in the resulting 3D matrices. The n-HA matrices contained in the dry state, 2.8+/- 0.1% (w/w) of HA. The use of n-HA in the dry form instead of a n-HA suspension, induced large aggregates inside the matrices. The 3D matrices in the presence of n-HA are porous (Figure 2) with pore sizes controlled by the patented process.

Discs of 4 mm in diameter of 3D porous matrices with or without n-HA (composite scaffold) and previously seeded or not with human adipose derived mesenchymal stem cells (ADSCs) were then evaluated in two mice models.

Orthotopic new bone formation on calvariae site of athymic mice revealed that only the polysaccharide-based matrices associated with n-HA (composite scaffold) induced formation of a mineralized tissue in nude mice. The porous

matrices without n-HA do not induce any mineralization within 60 days. The orthotopic new bone formation was observed with composite matrices in absence of human mesenchymal stem cells, and even if the scaffold moved out of the bone defect (Figure 3B). The mineralization occured four weeks after implantation and increased with time (Figure 3C). Histological examination (Goldner's trichrome staining) revealed a fibrous tissue formed when polysaccharide-based matrices without n-HA were implanted, whereas the composite polysaccharide scaffold provides an efficient scaffold for local production of collagen network within the matrices.

Since the n-HA matrix (composite scaffold) was found to induce mineralization outside the bone defect, the inventors next examined its potency to stimulate ectopic bone formation. They observed that implantation of matrices without n-HA did not form any mineralized tissue at day 60. In contrast, implantation of n-HA matrices (composite polysaccharide scaffold of the invention) in subcutaneous site lead to the formation of a dense mineralized tissue (Figure 4A and 4B) four weeks after implantation and without ADSCs seeding. The mineralization increased with time. Quantification indicated that the TMD of the calcified tissue was about 420 mg/cm^3 and close to the density of the implanted composite matrix in orthotopic site (Figure 4C) 60 days after implantation. Histological analysis on undecalcified (Figure 4D1) and decalcified (Figure 4D2) sections of the ectopically induced mineralized tissue revealed that n-HA matrices (composite polysaccharide scaffold MATRI+) stimulated a dense collagen network and blood vessel formation as well as the recruitment of osteoblast-like cells (Figure 4D3). To visualize the level of calcification in the newly formed tissue, sections of n-HA/scaffold were stained according to Von Kossa technique at day 30 and day 60 (Figure 4E). Controls were performed on the paraffin-embedded composite polysaccharide. This staining showed a well-calcified tissue of n-HA/scaffold that increases with time of implantation. To the knowledge of the inventors, no material so far in the absence of stem cells or growth factors, was able to give this effect.

The inventors further investigated for comparison the role of n-HA alone on non-osseous site. For this purpose, they proceed to the implantation of n-HA alone in subcutaneous site. After 15 days and 30 days, they only observed a classical reaction to a foreign body. Indeed, the histological examination of undecalcified section

(Cyanine Solochrome staining) of non-osseous site implanted with n-HA alone did not show the presence of any mineralized tissue. Implantation of n-HA alone hence did not lead to the formation of a mineralized tissue.

The inventors have thus shown that the porous composite polysaccharide scaffold of the invention provides unexpected results by stimulating mineralized tissue formation in osseous site, as well as in non-osseous site, in the absence of stem cells or growth factors.

Example 2 : Implantation of the scaffold according to the invention in a non osseous site in mice and osseous site in rat.

Materials and Methods

Nanohydroxyapatite and scaffold according to the invention were prepare as described in Example 1. The inventors assessed the implantation of said scaffold in animal. Both the procedure and the animal treatment complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The studies were carried out in accredited animal facilities at the University of Bordeaux Segalen, under authorization (N°: 3300048 of the Ministere de l'Agriculture, France) and were approved by the Animal Research Committee of Bordeaux University.

Non-osseous implantation in mice: ectopic bone formation analysis

The two different formulations of scaffolds: disk-shaped matrices without n-HA (Group 1) and the composite scaffold containing n-HA (MATRI+) (Group2) (cylinders of 4 mm diameter and 6 mm depth) were inserted into subcutaneous pockets created in the dorsum of the 12-week-old Balb/c mice weighing 25–30 g (Charles River Laboratories, France). Samples were retrieved after 15, 30 and 60 days of implantation and treated for micro-CT and histological analysis. Eight samples were used for histological observation and micro-CT in each group.

Osseous implantation in rats: orthotopic new bone formation analysis

Medial holes, 5 mm diameter and 6 mm depth were created in both left and right femoral condyles of Wistar rats weighing 150-200 g (Charles River

Laboratories, France) using trephine dental burr. Bone pieces were removed from the bone defect, the hole was rinsed with physiological solution (NaCl 0.9 % (w/v) before introducing the scaffold within the defect. The two different scaffold formulations (matrices without n-HA and composite scaffold containing n-HA) were implanted into each bone defect. A control experiment without scaffold was also conducted. Implants were retrieved 15, 30, 60 and 90 days after surgery and treated for micro-CT and histological analysis. Six samples were used for micro-CT and histological observation in each group.

Histological procedure

At the end of each implantation period, animals were euthanized by injecting an overdose of pentobarbital sodium (Nembutal®). Immediately afterwards, the implants and surrounding tissue were retrieved, fixed with 4% (w/v) paraformaldehyde in a 0.1 M phosphate buffer and scanned with micro-CT before histology. The samples were then prepared for histological analysis. One part was decalcified, dehydrated and embedded in paraffin. Thin sections (7 µm in thickness) were prepared and stained with hematoxylin and eosin and with Goldner's Trichrome for osteoid staining. The other part were dehydrated in a graded series of ethanol, and then embedded with methylmethacrylate, which was subsequently polymerized. Ten to 15 µm transverse sections were made using a modified diamond blade microtome (Leica Microsystems SP1600, Rijswijk, The Netherlands), with four sections obtained from each implant. Sections were stained with Goldner's trichrome, Von Kossa, and observed using a Nikon Eclipse 80i microscope. Pictures were generated using a DXM 1200 C (Nikon) CCD camera.

Micro-computed tomography (micro-CT)

Micro-CT was used to develop three-dimensional images of the implants and surrounding tissue; these models were used to quantify the bone formation at each implant site. An *ex vivo* General Electric (GE) micro-CT (Explore LP Locus, General Electric), with a source voltage of 80 kV, a current of 60 µA, and 15 µm resolution, was used to acquire X-ray radiographs. *In vivo* micro-CT (General Electric) was performed with a source voltage of 150 mV, a current of 450 µA, and 45 µm resolution. After scanning, cross-sectional slices were reconstructed and 3D analyses

were performed using Microview software. Each scan result was reconstructed using the same threshold values to distinguish bone and air. Bone Mineral Content (BMC) and Bone Mineral density (BMD) volume were measured for each group and statistically analyzed using the Student's *t*-test.

Protein extraction from subcutaneous implants and ELISA analysis of osteogenic and angiogenic growth factors retained within the implants.

Subcutaneous implants retrieved after 2, 15, 30 and 60 days of implantation were crushed on ice with an electric crusher in PBS containing a cocktail of protease inhibitors (10 µg/ml Aprotinine (Sigma), 10 µg/ml Leupeptin (Sigma) and 1 mM (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) (Fluka). The lysates were then centrifuged at 16 000 rpm and 4°C for 20 min. The supernatant was collected and then frozen at -80°C for ELISA analysis. Quantification of the protein was performed using bicinchoninic acid (BCA) protein assay kit (Thermoscientific) described by Smith PK *et al.* (1985). Absorbance was read at 550 nm. There were eight matrices without n-HA (Group 1) and composite scaffold MATRI+ containing n-HA samples (Group 2), respectively for each time of implantation. The amounts of $VEGF_{165}$ and BMP2 retained within the two different formulations of implants were quantified with the mouse VEGF immunoassay kit (MMV00, Quantikine ®, R&D sytems), and BMP-2 immunoassay kit (DBP200, Quantikine ®, R&D sytems), respectively.

X-ray diffraction analysis

Subcutaneous implants of matrices without n-HA and composite scaffold MATRI+ containing n-HA were retrieved after 15, 30 and 60 days of implantation. In order to obtain a fine powder without any organic tissues, they were treated with bleach for 2 hours at room temperature and then centrifuged to keep only the pellet. Structural properties were explored by X-ray diffraction (XRD) using PANalytical X'pert MPD diffractometer (Bragg Brentano t-t geometry) equipped with a secondary monochromator and uses a copper radiation (mean λ= 1,5418 A°), the working tension and intensity were 40 kV and 40 mA, respectively.

Samples were placed on a single-crystalline wafer sample holder made of silicium. Diffractograms were all measured with the same parameters: angular range

from 8 to 80° (2t), step: 0,02°, measure time: one hour; Following X-ray diffraction (XRD) analysis of the material, phase identification through JCPDS-ICDD data (Diffract-Plus Eva Software, Bruker©) was compatible with a carbonated hydroxyapatite [Ca10(PO4)3(CO3)0,01(OH)1,3], displaying hexagonal lattice parameters (a = 9.3892 A° ; c = 6.9019 A°; α=β=90° and g=120°; space group:P63/m(176)).

Statistical analysis

All data were expressed as means ± standard deviation (SD) and were analyzed using standard analysis of Student's t-test. Differences were considered significant when $p \leq 0.05$ (a) or $p \leq 0.01$ (b).

Results

Two different scaffolds, matrices without n-HA (Group 1) and the composite scaffold MATRI+ containing n-HA (Group 2), were implanted in Balb/c mice for 15, 30 and 60 days. Micro-CT, quantification of mineralization (BMC and BMD analysis) and histological studies were performed for both groups. Implantation of matrices without n-HA did not form any mineralized tissue from day 15 to day 60, as showed by micro-CT (Figure 5A) and BMC and BMD quantification (Figure 5B). In contrast, implantation in subcutaneous site of matrices containing n-HA (without any cells and growth factors) lead to the formation of a dense mineralized tissue (Figure 5A) as quantified by BMC (Bone Mineral Content) and BMD (Bone Mineral Density) measured at each time (Figure 1B). The mineralization process starts at day 15 from the periphery of the scaffold (Figure 1A) and lead to a high and dense mineralized tissue after 60 days of implantation.

From histological data, the porous n-HA matrices exhibited favorable mineralized tissue responses at D15 and D60, as demonstrated by von Kossa staining of undecalcified sections of MATRI+ (Figure 6A), compared to matrix without n-HA. Von kossa staining is high after 60 days of implantation of MATRI+, compared to the same scaffold at day 15. The n-HA matrices before implantation stained with von kossa revealed a slight staining, due to the presence of the nanohydroxyapatite within the scaffold (not shown). However, the staining is much lower than that observed after 30 and 60 days of implantation.

Moreover, Goldner staining performed 60 days after implantation on decalcified sections of MATRI+ (Figure 6B), revealed, a dense fibrous collagen tissue, mainly around the implant. Some collagen tissue penetrate within the scaffold, exhibiting some lining osteoblast-like cells indicated by white arrows, in contact with the scaffold and numerous vessels marked by black arrows on the histological picture. No inflammatory event was detectable with both scaffolds, whatever the time of implantation.

The XRD patterns of powder of n-HA matrices before implantation (D0) or retrieved at day 15 (D15) revealed specific peaks of hydroxyapatite at D15 on the spectrum (Figure 7A). Peaks of Halite (H), probably due to the treatment of the samples with bleach, were observed in all spectra. The XRD patterns obtained at day 30 and day 60 were similar than those observed at D15 for both groups (data not shown).

The inventors also explored whether the n-HA matrices compared to matrices without n-HA could interact with endogeneous osteogenic and angiogenic growth factors. They have tested two major growth factors that play a fundamental role in angiogenesis and osteogenesis, the isoform $VEGF_{165}$ and BMP2, an osteoinductive factor that could, by itself, induces mineralization and bone formation. Two days of implantation, corresponding to the inflammatory phase observed following material implantation, both samples retained the two growth factors but to a different extent. Strikingly, the amount of BMP2 retained on MATRI+ is 1.41 pg /µg protein extracted from the samples, while the matrix without n-HA retained only 0.12 pg /µg protein. For $VEGF_{165}$, the amount retained in MATRI+ and matrix without n-HA are 0.089 pg/µg protein and 0.055 pg/µg protein, respectively. With time of implantation, and during the formation of the dense mineralized tissue, the concentration of BMP2 (Figure 8A) and $VEGF_{165}$ (Figure 8B) decreased in both groups, compared to data obtained after 2 days, but remains significantly higher in the MATRI+ group after 30 and 60 days of implantation, compared to matrix without n-HA.

The scaffolds, matrices without n-HA (Group 1) and the composite scaffold MATRI+ containing n-HA (Group 2), were implanted in a critical size bone defect of 5 mm diameter and 6 mm depth in the femoral condyle of rats, for 15, 30 and 90 days. Micro-CT, quantification of mineralization (BMC and BMD analysis) and

histological analysis were performed for both groups. As showed by micro-CT, matrices with n-HA (MATRI+) (Figure 9A) formed within the bone defect, a highly dense mineralized tissue, compared to matrix without n-HA. Mineralization increases with time of implantation as shown by quantification analysis of the BMD and BMC (Figure 9B) from day 15 to day 90 of implantation. BMC and BMD in the control group (empty) remain lower than in the other groups, whatever the time of implantation.

Histological data after 90 days of implantation confirmed, a high staining by von Kossa of the matrices with n-HA (MATRI+) compared with the matrix alone without n-HA or the empty group (Figure 10A). Goldner staining evidenced a fibrous tissue in the empty bone defect, while bone formation was enhanced within the MATRI+ implant after 90 days of implantation and occurred in direct contact of the MATRI+ implant (Figure 10B).

CLAIMS

1. A method for preparing a porous polysaccharide scaffold comprising the following steps:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii),

wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably nano-hydroxyapatite.

2. The method according to claim 1 wherein the porogen agent is selected in the group consisting of sodium chloride, calcium chloride, ammonium carbonate, ammonium bicarbonate, calcium carbonate, sodium carbonate, and sodium bicarbonate and mixtures thereof.

3. The method according to claim 1 or 2 wherein the weight ratio of the polysaccharide to the porogen agent is in a range from 1:50 to 50:1, preferably from 1:30 to 30:1, preferably from 1:12 to 12:1, preferably 12:14.

4. A method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, and one cross-linking agent,

b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b),

wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite preferably nano-hydroxyapatite.

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

5. The method of any one of claims 1 to 4, wherein said polysaccharide is selected from the group consisting of dextran, pullulan, agar, alginic acid, starch, hyaluronic acid, inulin, heparin, fucoidan, chitosan and mixtures thereof .

6. The method of any one of claims 1 to 5, wherein said polysaccharide is a mixture of pullulan/dextran in a ratio in a range from 95:5 to 5:95, preferably in a ratio of 75:25 (w/w).

7. The method of any one of claims 1 to 5, wherein said polysaccharide is a mixture of pullulan/dextran/fucoidan in a ratio in a range from about 70:20:10 to about 50:20:30, preferably from about 70:20:10 to about 50:30:20, and most preferably in a ratio of about 73:22:5 (w/w).

8. The method according to any one of claims 1 to 7, wherein said cross-linking agent is selected from the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride ($POCl_3$), epichlorohydrin, formaldehydes, carbodiimides, glutaraldehydes, and mixtures thereof.

9. The method according to any one of claims 1 to 8, wherein said nano-hydroxyapatite is obtained from a solution of phosphoric acid, at a concentration comprised between 0.3 to 1M, preferably 0.6M, with a solution of calcium hydroxide, at a concentration comprised between 0.5 to 1.5M, preferably 1M and preferentially through chemical precipitation at room temperature.

10. The method according to claim 9, wherein the concentration of nano-hydroxyapatite in the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v).

11. A porous polysaccharide scaffold obtainable by the method according to any one of claims 1 to 10.

12. The porous polysaccharide scaffold of claim 11, wherein the size of the pores is comprised from about 1 μm to about 500 μm, preferably from about 10 μm to about 200 μm, and the porosity is from about 4% to about 75%, preferably from about 4% to about 50%.

13. A porous polysaccharide scaffold obtainable according to method of claim 1 to 10, for use for bone generation, preferably bone repair and/or bone development.

14. A porous polysaccharide scaffold obtainable according to method of claim 1 to 10, for use for stimulating ectopic mineralized tissue formation.

15. A porous polysaccharide scaffold obtainable according to method of claim 1 to 10, for use in the treatment of bone related disorders.

FIGURES

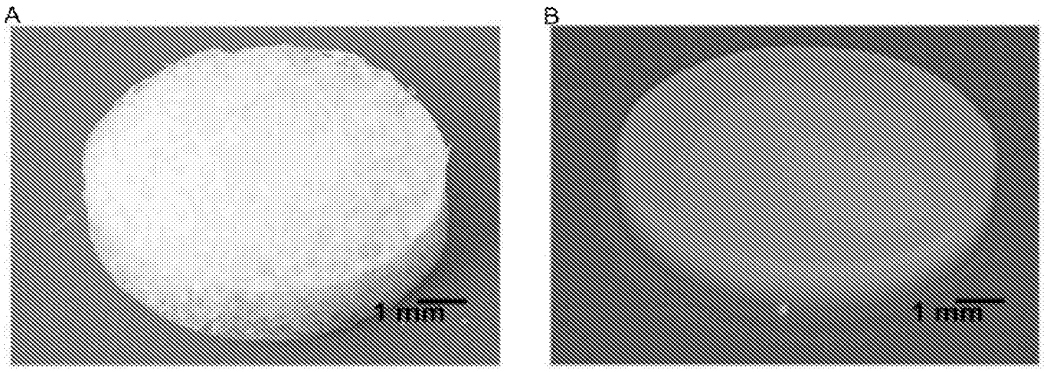


Figure 1

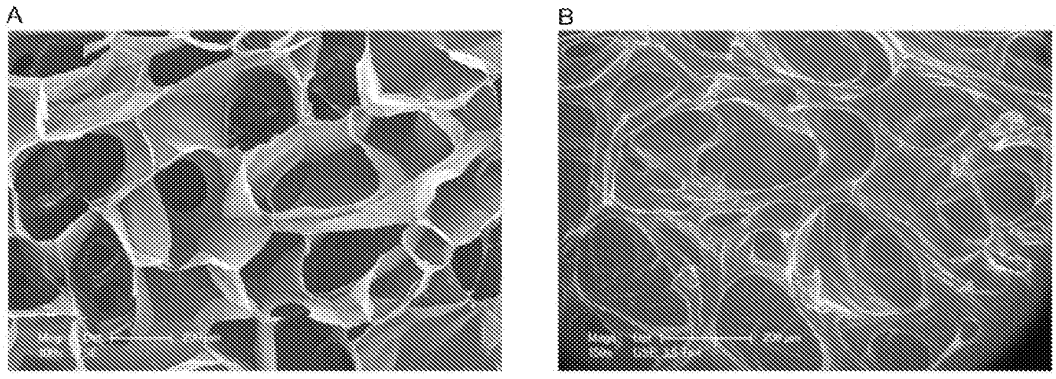


Figure 2

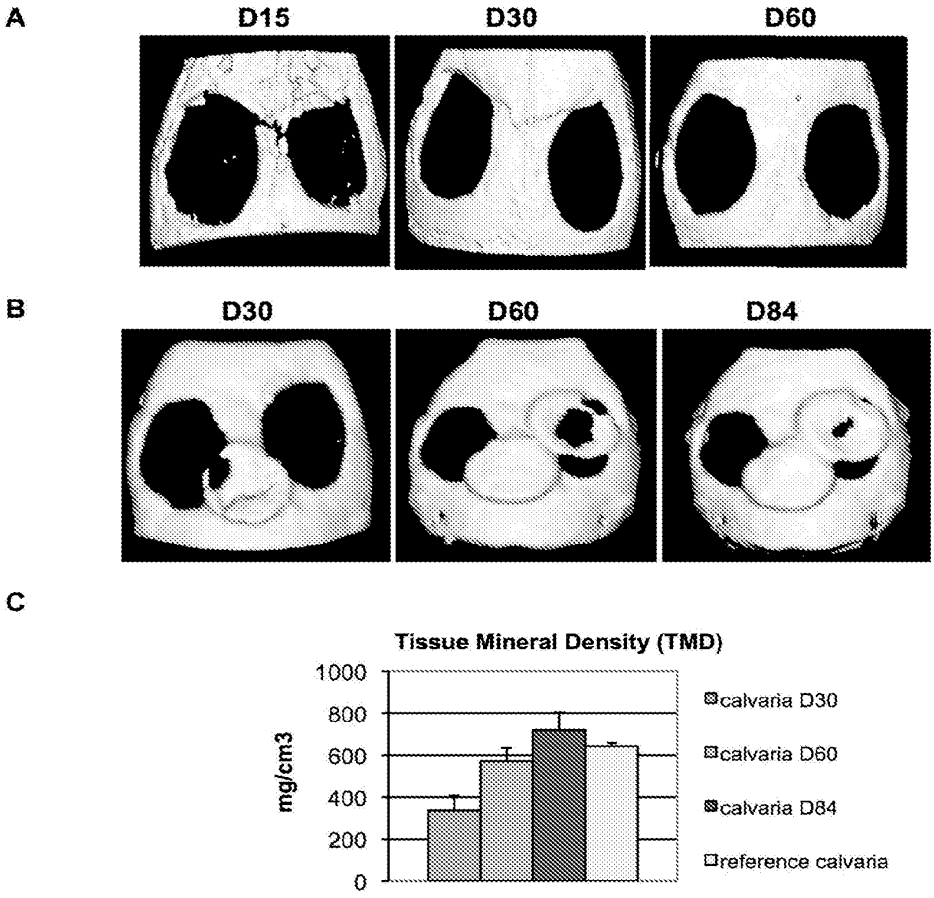

A
D15
D30
D60
B
D30
D60
D84
C
Tissue Mineral Density (TMD)
mg/cm3
1000
800
600
400
200
0
calvaria D30
calvaria D60
calvaria D84
reference calvaria

Figure 3

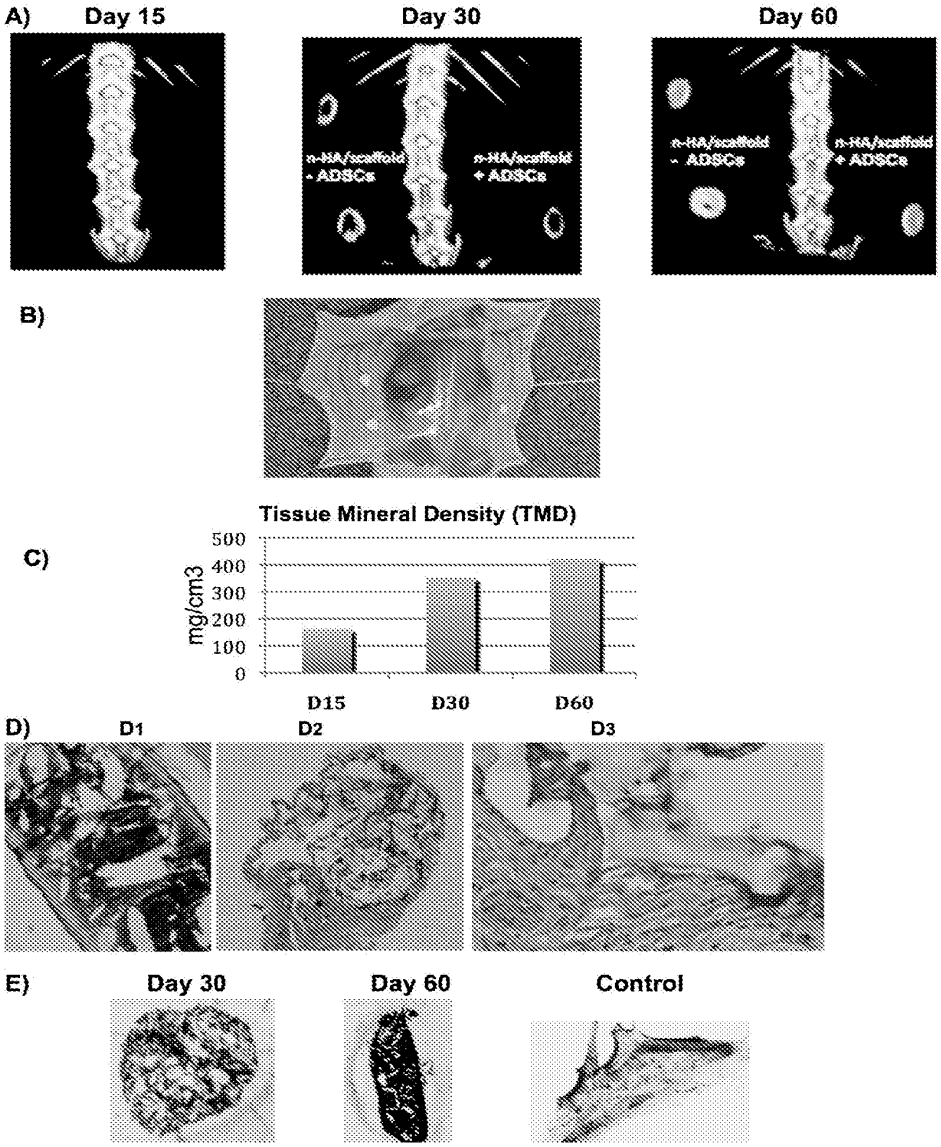

A)
Day 15
Day 30
Day 60
n-HA/scaffold
- ADSCs
n-HA/scaffold
+ ADSCs
n-HA/scaffold
- ADSCs
n-HA/scaffold
+ ADSCs
B)
C)
Tissue Mineral Density (TMD)
mg/cm3
500
400
300
200
100
0
D15
D30
D60
D)
D1
D2
D3
E)
Day 30
Day 60
Control

Figure 4

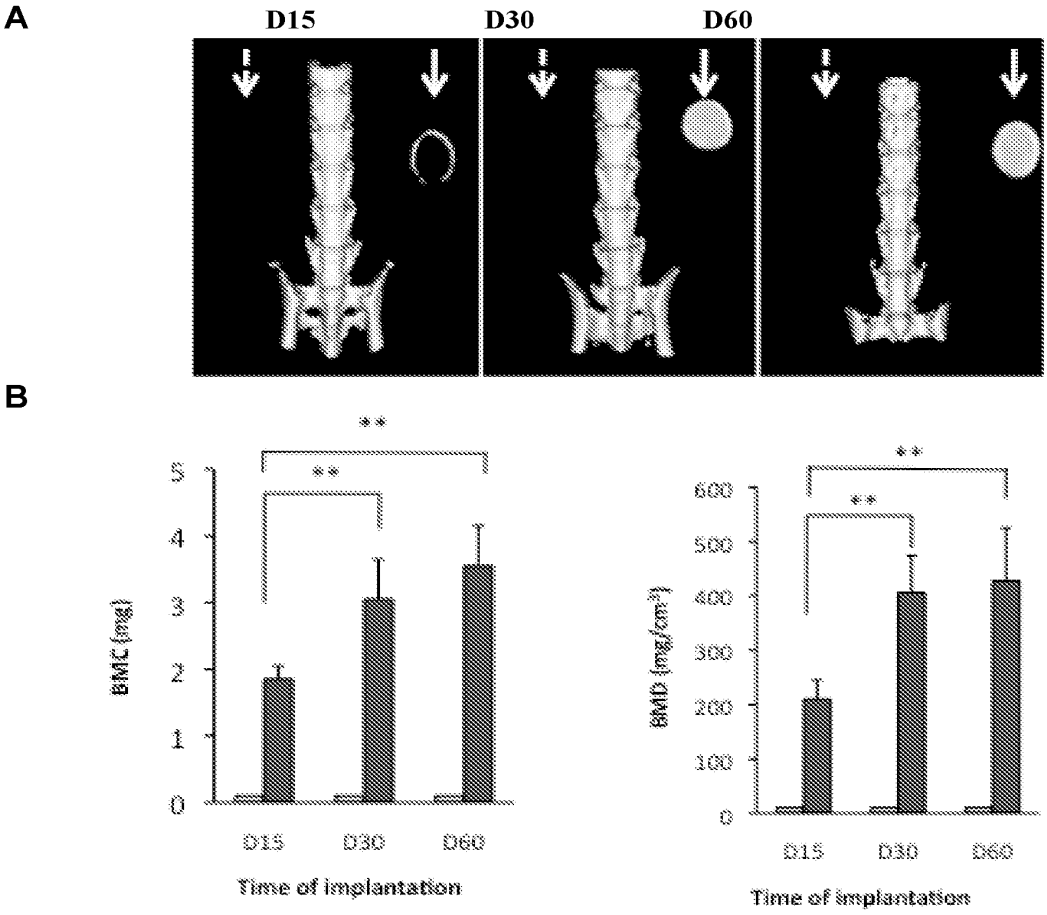

A
D15
D30
D60
B
**
**
BMC (mg)
0
1
2
3
4
5
D15
D30
D60
Time of implantation
**
**
BMD (mg/cm²)
0
100
200
300
400
500
600
D15
D30
D60
Time of implantation

Figure 5

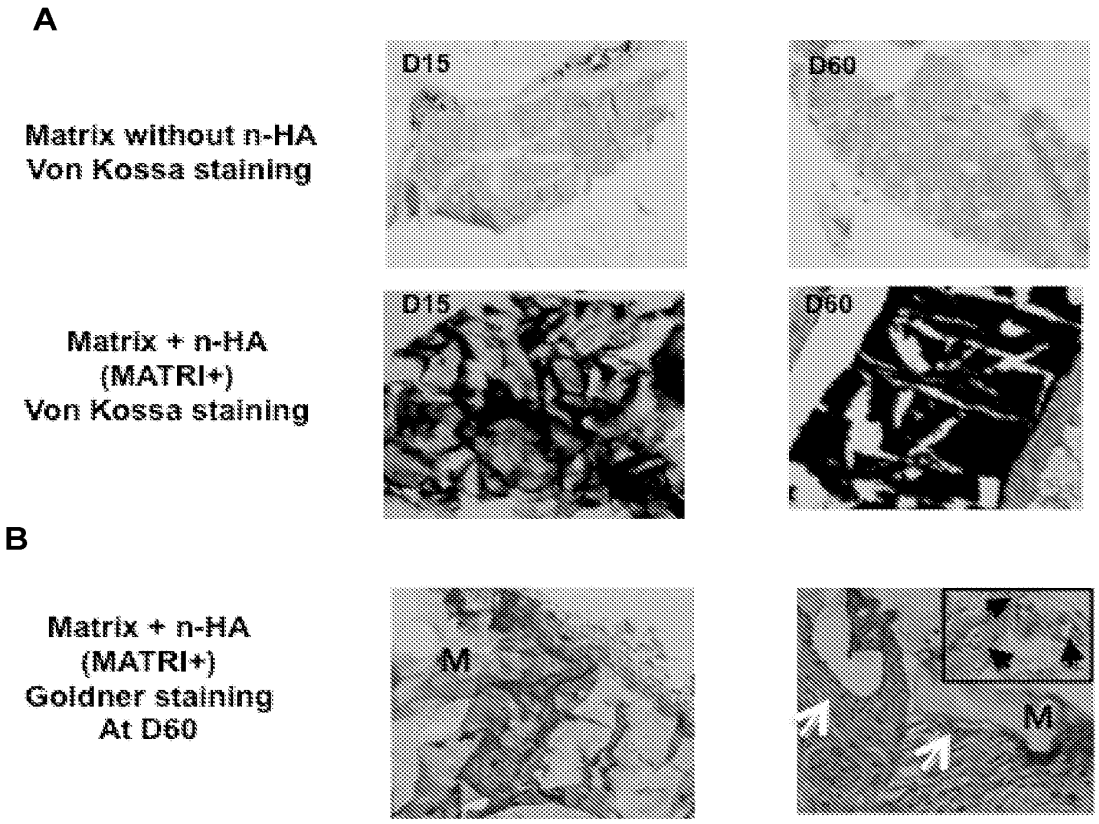
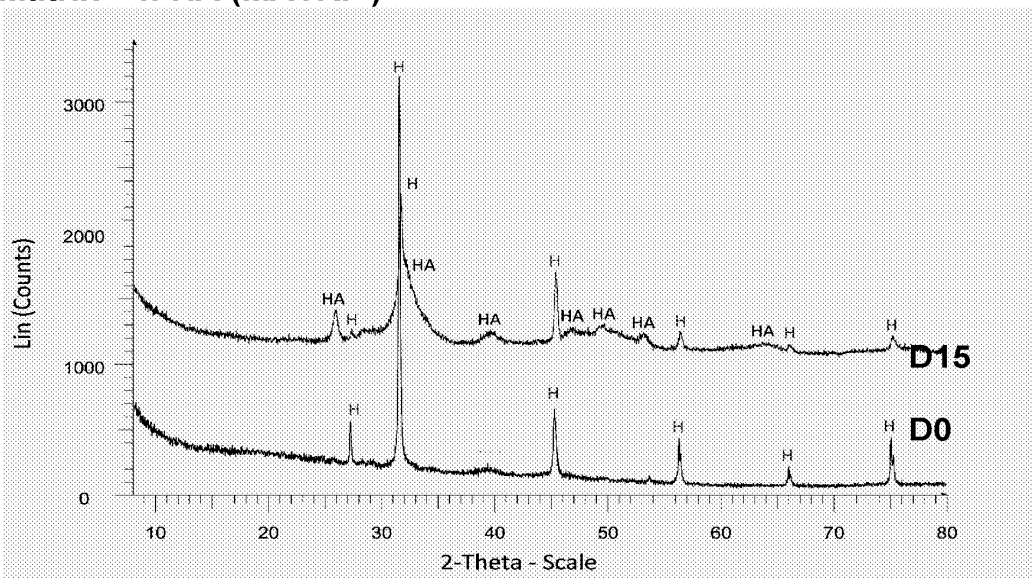

A
Matrix without n-HA
Von Kossa staining
D15
D60
Matrix + n-HA
(MATRI+)
Von Kossa staining
D15
D60
B
Matrix + n-HA
(MATRI+)
Goldner staining
At D60
M
M

Figure 6

A : Matrix + n-HA (MATRI+)



B : Matrix without n-HA

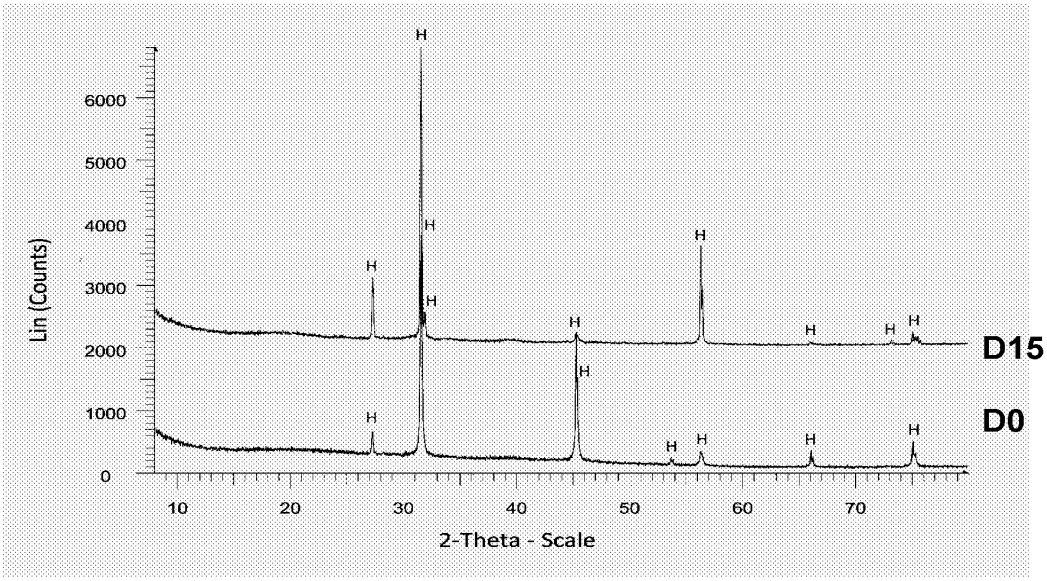


Figure 7

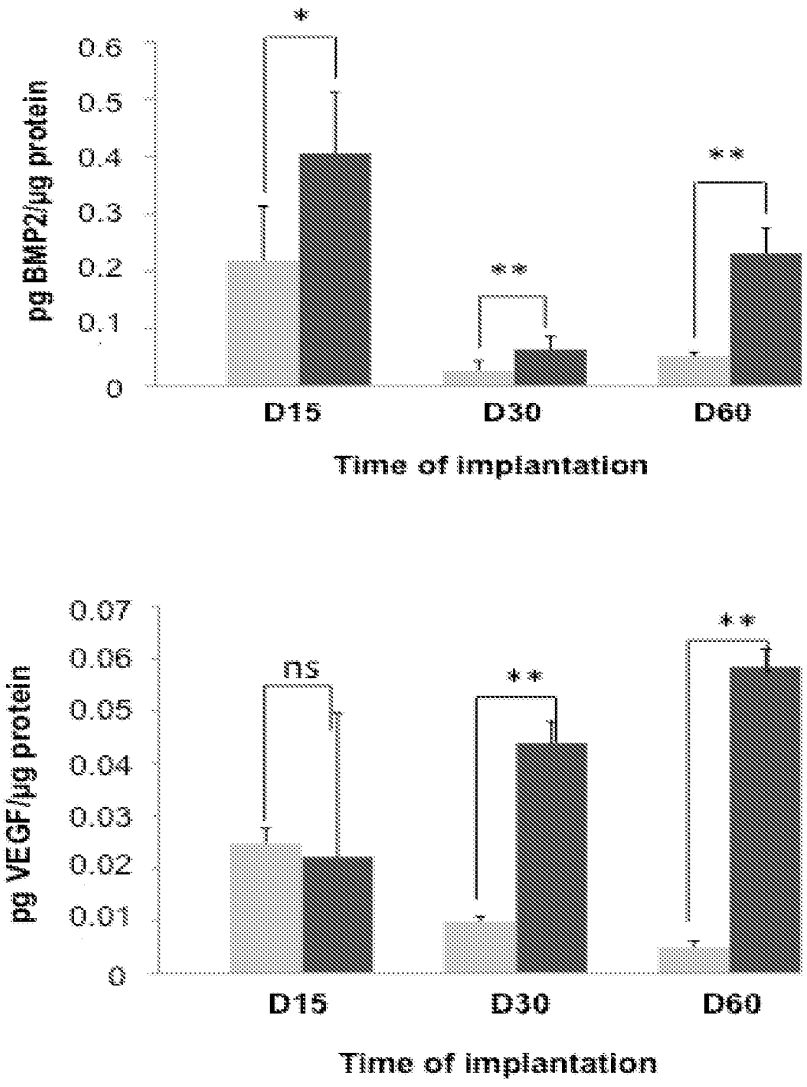
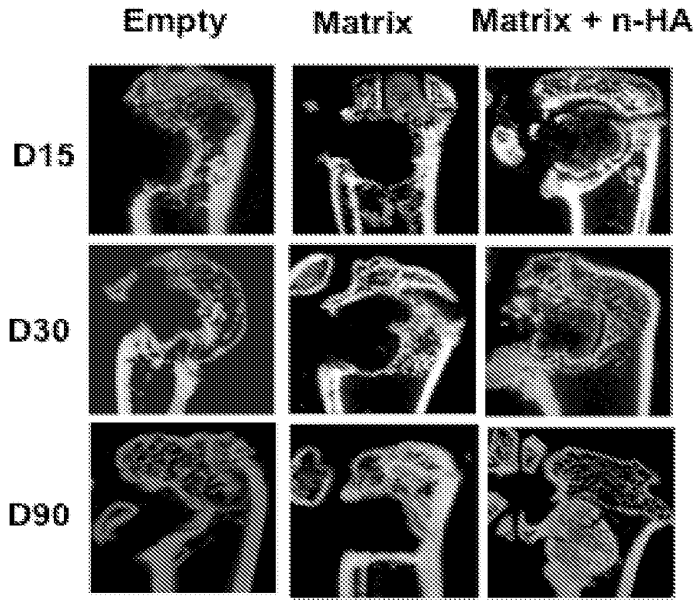

pg BMP2/µg protein
0.6
0.5
0.4
0.3
0.2
0.1
0
*
**
**
D15
D30
D60
Time of implantation
pg VEGF/µg protein
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
ns
**
**
D15
D30
D60
Time of implantation

Figure 8

A


Empty
Matrix
Matrix + n-HA
D15
D30
D90

B

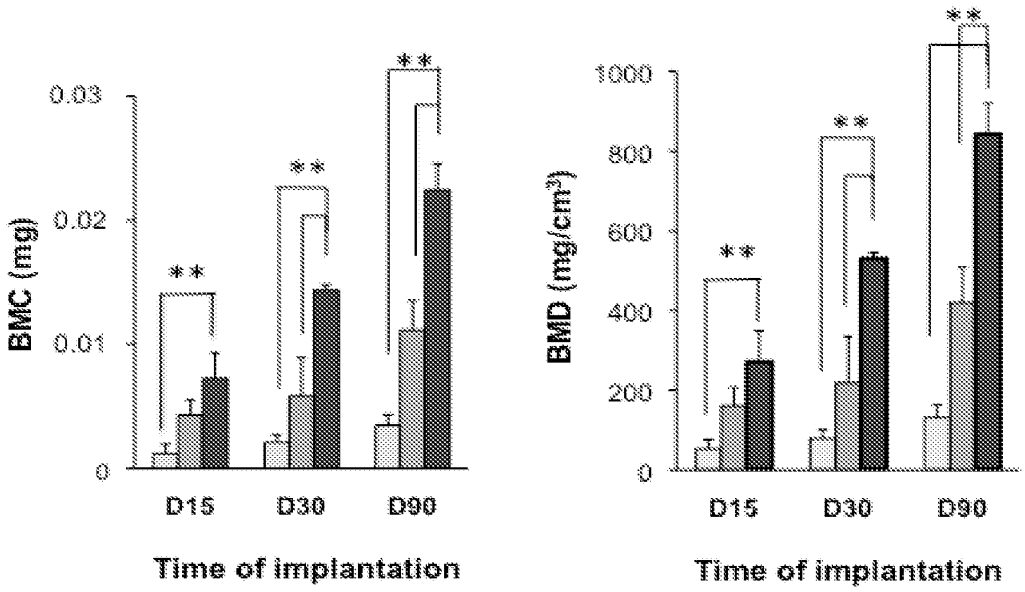

BMC (mg)
0.03
0.02
0.01
0
**
**
**
D15
D30
D90
Time of implantation
BMD (mg/cm³)
1000
800
600
400
200
0
**
**
**
D15
D30
D90
Time of implantation

Figure 9

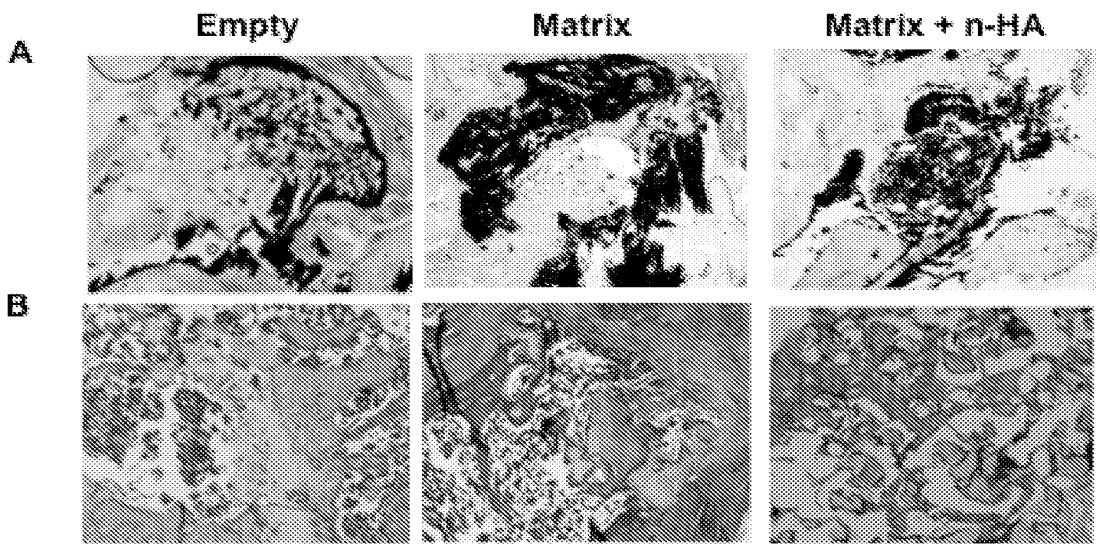


Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/064924

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/20 A61L27/46 A61L27/56
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/153814 A1 (LIAO CHUN-JEN [TW] ET AL) 13 July 2006 (2006-07-13) paragraph [0028] - paragraph [0035] paragraphs [0038], [0042]; claims; examples	1,5, 11-15
X	WO 2009/047346 A1 (INST NAT SANTE RECH MED [FR]; UNIV PARIS 7 DENIS DIDEROT [FR]; LE VISA) 16 April 2009 (2009-04-16) page 3, lines 11-23 pages 5,6 page 14, lines 16-19; claims; examples	2,3,6-10
X	WO 2009/047347 A1 (INSERM INST NAT DE SANTE ET DE [FR]; UNIV PARIS 7 DENIS DIDEROT [FR];) 16 April 2009 (2009-04-16) page 3, line 24 - page 4, line 8; claims; examples	4

-/--

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2011	20/12/2011

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIUYUN, YUBAO, LI, JIANGUO: "preparation and properties of a novel bone repair composite : nano HAP/chitosan/CMC", J. MATER SCI: MAT MED, vol. 19, 1 January 2008 (2008-01-01), pages 981-987, XP002608050, DOI: 10.1007/s10856-007-3208-1 the whole document -----	1-15
A	KONG, GAO, CAO, GONG, ZHAO, ZHANG: "preparation and characterization of nano-hydroxyapatite/chitosan composite scaffolds", J. BIOMED MAT RES, vol. 75A, no. 2, 1 November 2005 (2005-11-01), pages 275-282, XP002608051, DOI: 10.1002/jbm.a.30414 the whole document -----	1-15

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/064924

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006153814 A1	13-07-2006	TW I306896 B	01-03-2009
		US 2006153814 A1	13-07-2006
WO 2009047346 A1	16-04-2009	CA 2701858 A1	16-04-2009
		CN 101848738 A	29-09-2010
		EP 2203194 A1	07-07-2010
		JP 2011500118 A	06-01-2011
		KR 20100080924 A	13-07-2010
		US 2010221303 A1	02-09-2010
		WO 2009047346 A1	16-04-2009
WO 2009047347 A1	16-04-2009	CA 2704738 A1	16-04-2009
		CN 101820929 A	01-09-2010
		EP 2200671 A1	30-06-2010
		JP 2011500119 A	06-01-2011
		KR 20100087317 A	04-08-2010
		US 2010221301 A1	02-09-2010
		WO 2009047347 A1	16-04-2009

Form PCT/ISA/210 (patent family annex) (April 2005)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
MORRISON & FOERSTER LLP LEW
Attn. Smith, Michael G.
12531 High Bluff Drive, Suite 100
San Diego CA 92130-2040
ETATS-UNIS D'AMERIQUE

DOCKETED: RESP TO ISR
REMINDER: 1/30/10
FINAL DUEDATE: 2/28/10

Applicant's or agent's file reference 643982000141	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2009/055256	International filing date *(day/month/year)* 27/08/2009
Applicant Hyperion Therapeutics	Date of mailing *(day/month/year)* 30/12/2009

DOCKETED: RESP TO NO / CH. II DEMANDS.
REMINDER: 3/29/10
FINAL DUEDATE: 6/29/10

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Monika Langerova

Form PCT/ISA/220 (July 2009) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annex B).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, International Phase, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet or sheets containing a complete set of claims in replacement of all the claims previously filed must be submitted.

Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively in Arabic numerals (Section 205(a)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annex B).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, International Phase, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet or sheets containing a complete set of claims in replacement of all the claims previously filed must be submitted.

Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively in Arabic numerals (Section 205(a)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
643982000141		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2009/055256	27/08/2009	29/08/2008
Applicant		
Hyperion Therapeutics		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 1
      [ ] as suggested by the applicant
      [X] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

national application No
PCT/US2009/055256

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01), pages 1117-1121, XP009127277 ISSN: 0031-3998	30-33
Y	the whole document	1-29
	-/--	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2009	30/12/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Moreno de Vega, C

1

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MACARTHUR ROBERT B ET AL: "Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers" MOLECULAR GENETICS AND METABOLISM, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 81, no. Suppl.1, 1 April 2004 (2004-04-01), pages S67-S73, XP009127291 ISSN: 1096-7192 the whole document	1-33
Y	TANNER L M ET AL: "Nutrient intake in lysinuric protein intolerance" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER ACADEMIC PUBLISHERS, DO, vol. 30, no. 5, 21 June 2007 (2007-06-21), pages 716-721, XP019548954 ISSN: 1573-2665 page 716 - page 717	1-33
X	LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01), page 91, XP009127344 ISSN: 0141-8955	1-5, 15-17, 19-22, 30-33
Y	the whole document	1-33

1

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2009/055256	27.08.2009	29.08.2008

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
Hyperion Therapeutics

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

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

2. **FURTHER ACTION**

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

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

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

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Moreno de Vega, C Telephone No. +49 89 2399-7486

Form PCT/ISA/237 (Cover Sheet) (April 2005)

Box No. I Basis of the opinion

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

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

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

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

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

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

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

5. Additional comments:

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

1. Statement

Novelty (N)	Yes:	Claims	6-14, 18, 23-29
	No:	Claims	1-5, 15-17, 19-22, 30-33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-33
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

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

Reference is made to the following documents:

D1 SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01) , pages 1117-1121, XP009127277 ISSN: 0031-3998

D4 LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01) , page 91, XP009127344 ISSN: 0141-8955

1 Claims 12-18 and 26-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT.

The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Document D1 discloses the study of the metabolic changes caused by benzoate and phenylacetate and their pharmacokinetics in the treatment of an urea cycle disorder, the lysinuric protein intolerance, and that 54% of the single phenylacetate dose was excreted in urine as phenylacetylglutamine in 24 hours after the load. This document appears to be novelty destroying for claims 30-33.

3 Document D2 discloses the use of HPN-100, a PBA (phenylbutyrate) prodrug, in the treatment of urea cycle disorders and the assessment of its metabolites and the urinary excretion of nitrogen by phenylacetylglutamine measurement. This document appears to be novelty destroying for claims 1-5, 15-17, 19-22 and 30-33.

Thus, claims 1-5, 15-17, 19-22 and 30-33 do not meet the requirements of Article 33(2) PCT.

4 Considering D2 as the most relevant prior art, the technical problem to be solved by claims 6-14, 18 and 23-29 is the provision of a method for determining an effective dosis of HPN-100 for a patient in need of treatment for a nitrogen retention disorder. The solution proposed by said claims is based on a treatment with a known compound which is prodrug of compounds known from D1 to have an excretion rate as disclosed in the claims, and therefore cannot be considered to be inventive.

Thus, claims 1-33 do not meet the requirements of Article 33(3) PCT.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

BNSDOCID: <XS_____2006100103CF_I_>

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)
(PCT Rule 44bis.1(c))

To:

079532-8003.WO00
PDM/CDK

MORRIS, Patrick D.
Perkins Coie LLP
P.O. Box 1208
Seattle, Washington 98111-1208
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 10 April 2014 (10.04.2014)	
Applicant's or agent's file reference 795328003WO	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/028620	09 March 2012 (09.03.2012)	30 September 2011 (30.09.2011)

Applicant
HYPERION THERAPEUTICS, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 82 70

Authorized officer
Philippe Bécamel

e-mail: pt03.pct@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 795328003WO	**FOR FURTHER ACTION** See item 4 below	
International application No. PCT/US2012/028620	International filing date *(day/month/year)* 09 March 2012 (09.03.2012)	Priority date *(day/month/year)* 30 September 2011 (30.09.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant HYPERION THERAPEUTICS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 April 2014 (01.04.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 JUN 2012
Applicant's or agent's file reference 795328003WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/028620	09 March 2012	30 September 2011

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2

Applicant SCHARSCHMIDT, BRUCE

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

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

2. **FURTHER ACTION**

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

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

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

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	04 June 2012	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Box No. I Basis of this opinion

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

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

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

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

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

   a. (means)

   ☐ on paper

   ☐ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

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

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

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

1. Statement

Novelty (N)	Claims	8	YES
	Claims	1-7, 9-12	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-7 and 9-12 lack novelty under PCT Article 33(2) as being anticipated by Scharschmidt et al. (hereafter Scharschmidt).

Regarding claim 1, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]);
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26. 1 umol/L), Para. [0201]).

Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0064]) comprising:

a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]);

Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]).

Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]).

Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPEA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]).

Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]).

Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]).

Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects is between 26 to 35 umol/L, Para. [0094]).

Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising:
c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and
e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claim 8 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al.(hereafter Scharschmidt) in view of Ennis et al. (hereafter Ennis).

Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). It would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with lethal urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16).

Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

079532-8003.WO00
PDM/CDK

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247
USA

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Rule 71.1)

Date of mailing (*day/month/year*)	04 SEP 2013

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
79532.8003.WO00	

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US12/28620	09 March 2012 (09.03.2012)	30 September 2011 (30.09.2011)

Applicant

HYPERION THERAPEUTICS, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the *PCT Applicant's Guide*.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/ US	Authorized officer
Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	SAVITHA RAO Telephone No.

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 79532.8003.WO00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US12/28620	International filing date *(day/month/year)* 09 March 2012 (09.03.2012)	Priority date *(day/month/year)* 30 September 2011 (30.09.2011)

International Patent Classification (IPC) or national classification and IPC
IPC: **A61B 5/11**(2006.01);**A61K 31/192**(2006.01);**A61K 49/00**(2006.01),**A61P 13/00**
USPC: 424/9.2,514/568,600/322

Applicant
HYPERION THERAPEUTICS, INC.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 11 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4*bis*, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No.I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____ containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07 December 2013 (07.12.2013)	22 August 2013 (22.08.2013)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer SAVITHA RAO Telephone No.

Form PCT/IPEA/409 (cover sheet) (July 2011)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US12/28620

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:
   - ☒ the international application in the language in which it was filed.
   - ☐ a translation of the international application into English which is the language of a translation furnished for the purposes of:
     - ☐ international search (Rules 12.3(a) and 23.1(b)).
     - ☐ publication of the international application (Rule 12.4(a)).
     - ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a) and (b)).
2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
   - ☐ the international application as originally filed/furnished
   - ☒ the description:
     pages 1-30 as originally filed/furnished
     pages* NONE received by this Authority on _____
     pages* NONE received by this Authority on _____
   - ☒ the claims:
     pages NONE as originally filed/furnished
     pages* NONE as amended (together with any statement) under Article 19
     pages* 31-32 received by this Authority on 07 DECEMBER 2012 (07.12.2012)
     pages* NONE received by this Authority on _____
   - ☒ the drawings:
     pages 1-3 as originally filed/furnished
     pages* NONE received by this Authority on _____
     pages* NONE received by this Authority on _____
   - ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages _____
   - ☐ the claims, Nos. _____
   - ☐ the drawings, sheets/figs _____
   - ☐ the sequence listing (*specify*): _____
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and(*c-bis*)):
   - ☐ the description, pages _____
   - ☐ the claims, Nos. _____
   - ☐ the drawings, sheets/figs _____
   - ☐ the sequence listing (*specify*): _____
5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.4*bis*) and 70.2(e)).
6. ☐ Supplementary international search report(s) from Authority(ies) _____ has/have been received and taken into account in establishing this report (Rule 45*bis*.8(b) and (c)).

** If item 4 applies, some or all of those sheets may be marked "superseded."*

Form PCT/IPEA/409 (Box No. I) (July 2011)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US12/28620

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-12	YES
	Claims NONE	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-12	NO
Industrial Applicability (IA)	Claims 1-12	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)
Please See Continuation Sheet

Form PCT/IPEA/409 (Box No. V) (July 2011)

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

V. 2. Citations and Explanations:
Claim 1-12 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al. in view of Ennis et al.

Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising: a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]). Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0064]) comprising: a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon

Supplemental Box

ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]. Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising: a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]). Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]); Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]). Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]). Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl td-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPEA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]). Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]). Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]). Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects are between 26 to 35 umol/L, Para. [0094]). Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising: c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).
As such the teachings of Scharschmidt et al. clearly provides a person of ordinary skill in the art explicit suggestions and motivation to develop the instantly claimed methods. Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). it would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with leth'al urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16). Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

---------- NEW CITATIONS ----------

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 02 MAR 2009
Applicant's or agent's file reference 643982000140	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 09/30362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)
Applicant HYPERION THERAPEUTICS	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 740 14 35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	02 MAR 2009
Applicant's or agent's file reference 643982000140	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 09/30362	07 January 2009 (07.01.2009)	29 April 2008 (29.04.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570

Applicant HYPERION THERAPEUTICS

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

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

2. **FURTHER ACTION**

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

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

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

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 February 2009 (24.02.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

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

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:
   a. type of material
      - [ ] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [ ] on paper
      - [ ] in electronic form

   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [ ] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

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

1. Statement

Novelty (N)	Claims	1-29	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-29	NO
Industrial applicability (IA)	Claims	1-29	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0229948 A1 to Summar, et al. (hereinafter "Summar") in view of US 4,284,647 A to Brusilow, et al. (hereinafter "Brusilow-647").

Regarding claim 1, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary PAGN output taught in Brusilow-647, in order to determine the effective dosage of HPN-100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Regarding claim 2, Brusilow-647 further teaches the method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine (Fig. 3; col 4, ln 35-46).

Regarding claim 3, Summar further teaches the method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy (para [0029]).

Regarding claim 4, Summar further teaches the method of claim 1, wherein administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Summar does not explicitly teach achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; col 4, ln 64-68).

Regarding claim 5, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary phenylacetyl glutamine output and total urinary nitrogen (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary phenylacetyl glutamine output taught in Brusilow-647, in order to determine the effective dosage of HPN -100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Claims 6-8, 19-22 and 28 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of US 5,968,979 A to Brusilow (hereinafter "Brusilow-979").

Regarding claim 6, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach HPN-100 conversion to PAGN. However, Brusilow-979 teaches HPN-100 conversion to PAGN (col 4, ln 1-26, "n = 2"; col 5, ln 3-15; col 5, ln 29-35). It would have been obvious to one of ordinary skill in the art to calculate the dosage of HPN-100 based on a utilization efficiency for HPN-100 conversion into PAGN of about 60% to about 75%, in order to achieve effective plasma concentrations of phenylacetate for acetylation of glutamine, by routine experimentation, as Brusilow-979 teaches the intermediate formation of phenylacetate that produces PAGN by acetylation of glutamine (col 3, ln 3-7).

==========Continued in Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 7, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 6. Neither Summar nor Brusilow teaches a method wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100, in order to effectively deplete accumulated nitrogen via acetylation of glutamine, as taught in Brusilow-979 (col 3, ln 3-7), as the plasma level of glutamine would be likely to depend on the protein intake of the patient, as taught in Brusilow-979 (col 1, ln 41-45).

Regarding claim 8, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 7. Neither Summar nor Brusilow-979 teaches a method wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity. However, it would have been obvious to one of ordinary skill in the art to reduce the dosage to account for the patient's residual urea synthesis capacity, by routine experimentation, as urea synthesis would be likely to lesson the plasma nitrogen accumulation, as taught in Brusilow-979 (col 1, ln 27-34).

Regarding claim 19, Brusilow-979 teaches a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA (col 2, ln 25-34; col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach determining the AUC and Cmax for PBA when the patient receives the PBA prodrug. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), by comparing the AUC and Cmax for the prodrug with those when the patient receives an equimolar amount of PBA, by routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Regarding claim 20, Brusilow-979 further teaches the method of claim 19, wherein the PBA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claims 21 and 22, Brusilow-979 (col 2, ln 25-34; col 3, ln 42-59) and Summar (para [0035]) teach the method of claim 20. Neither Brusilow nor Summar teaches a method wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20% or by at least 30%. However, it would have been obvious to one of ordinary skill in the art to expect AUC for PBA exposure to be lower by 20-30% for PBA prodrug than with PBA, in order to treat UCD with minimum exposure to PBA, as taught in Brusilow-979 (col 2, ln 15-24), as the triglyceride of PBA would be likely to produce a stable drug level by gradual beta-oxidation of the prodrug, as taught in Brusilow-979 (col 2, ln 25-34).

Regarding claim 28, Brusilow-979 teaches a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach the AUC or Cmax of PBA. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug so that AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), through routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Claims 12-18 and 23-27 lack an inventive step under PCT Article 33(3) as being obvious over Brusilow-647 in view of Brusilow-979.

Regarding claim 12, Brusilow-979 teaches a method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug comprising administering to the patient the suitable dosage of the PAA prodrug (col 4, ln 1-26; col 3, ln 56-59). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output in a patient (col 2, ln 26-32; Fig 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output based on 60-75% convertion of the pro-drug, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, by the method taught in Brusilow-647, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN for a patient, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 13, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 14, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100, administered in fewer doses per day (col 3, ln 42-55; col 4, ln 1-26). Brusilow-979 does not teach administering two or three doses of HPN-100 per day. However, it would have been obvious to one of ordinary skill in the art to administer two or three doses of HPN-100 to the patient with clinically significant residual urea synthetic capacity, in order to reduce plasma ammonium to normal levels, as the urea synthetic capacity would be likely to aid in the depletion of nitrogen, as taught in Brusilow-979 (col 1, ln 27-34), thus reducing the number of doses per day of HPN-100 required to be administered to the patient.

==========Continued in Next Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Prior Supplemental Box:

Regarding claim 15, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 by the method taught in Brusilow-647, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 16, Brusilow-979 teaches the method of claim 15 (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach determining the urinary PAGN. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to reduce the amount of HPN-100 based on the increase in the amount of urinary PAGN caused by the transition, in order to effectively treat nitrogen-retention disorders, by routine experimentation, as a correlation between urinary PAGN output and HPN-100 is taught in Brusilow-979 (col 5, ln 3-15; ln 29-35).

Regarding claim 17, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to gradually transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100 in small amounts, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 in depleting waste nitrogen as PAGN, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 18, Brusilow-979 teaches a method of treatment with HPN-100 (col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to initiate treatment with HPN-100 in a step-wise fashion and increase the amount of HPN-100 gradually, by monitoring the urinary PAGN based on 60-75% convertion by the method taught in Brusilow-647, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the maintenance dose of HPN-100 effective for the treatment of nitrogen-retention disorders, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and HPN-100 administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 23, Brusilow-647 teaches a method to determine the nitrogen elimination capacity of a patient having a nitrogen retention disorder, being treated with a nitrogen scavenging drug (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine"). Brusilow-647 does not teach a method to determine a suitable dietary protein level for a patient. However, it would have been obvious to one of ordinary skill in the art to use the method taught in Brucilow-647 to determine the patient's endogenous nitrogen elimination capacity with and without the nitrogen scavenging drug, in order to determine the amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, through routine experimentation, since the dietary protein intake would be likely to influence the nitrogen elimination capacity of the patient, as taught in Brucilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 24, Brusilow-979 further teaches the method of claim 23, wherein the nitrogen scavenging drug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 25, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 24, wherein Brusilow-979 teaches the selected dosage of HPN-100 (col 4, ln 54-58). Neither Brusilow-647 nor Brusilow-979 teaches a dosage of HPN-100 of up to about 19 grams per day. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen, as PAGN as taught in Brusilow-979 (col 5, ln 3-15), by routine experimentation, as the patient's inherent ability to process nitrogen and the dietary protein intake would be likely to influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Regarding claim 26, Brusilow-979 teaches a method to treat a patient with a PBA prodrug, comprising administering HPN-100 to a subject having HE or UCD (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26; col 4, ln 54-58). Brusilow does not teach a daily dose in excess of 19 g per day of the prodrug. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, since the patient's inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

==========Continued in Next Supplemental Box==========

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 27, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 26. Neither Brusilow-647 nor Brusilow-979 teaches a daily dose of HPN-100 is between about 199 and about 57 g. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN, as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, as the patients inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Claims 9-11 and 29 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of Brusilow-647 and further in view of Brusilow-979.

Regarding claim 9, Summar teaches a method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder (para [0022],"glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not explicitly teach determining the patient's residual urea synthesis capacity or dietary intake or estimating the urinary PAGN output. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output for a patient based on 60-75% convertion of the pro-drug, by the method taught in Brusilow-647, by taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15; col 5, ln 29-35).

Regarding claim 10, Summar further teaches the method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof (para [0022]).

Regarding claim 11, Summar further teaches the method of claim 9, wherein the PAA prodrug is HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)").

Regarding claim 29, Brusilow-979 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26) and Summar (para [0035]) teach the method of claim 28, wherein Summar further teaches that administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Neither Brusilow-979 nor Summar explicitly teaches achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; ln 64-68).

Claims 1-29 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 643982000140	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 09/30362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)	(Earliest) Priority Date *(day/month/year)* 29 April 2008 (29.04.2008)
Applicant HYPERION THERAPEUTICS		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

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

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

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 4

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/30362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
US WEST(PGPB,USPT,EPAB,JPAB), Google Scholar, Dialog PRO (Engineering)
ammonia scavenging, accumulation, retention, hepatic encephalopathy, urea cycle disorder, phenylacetyl glutamine, PAGN, HPN-100, phenyl butyrate, glyceryl tri-(4-phenyl butyrate)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0229948 A1 (SUMMAR, et al.) 18 November 2004 (18.11.2004), para [0022], [0029], [0035]	1-11, 19-22, 28, 29
Y	US 4,284,647 A (BRUSILOW, et al.) 18 August 1981 (18.08.1981) col 2, ln 26-32; Fig. 3; col 4, ln 35-46.	1-5, 9-18, 23-27, 29
Y	US 5,968,979 A (BRUSILOW) 19 October 1999 (19.10.1999), col 1, ln 27-34; col 1, ln 41-45; col 2, ln 25-34; col 3, ln 3-7; col 3, ln 42-59; col 4, ln 1-26; col 4, ln 54-58; col 5, ln 3-15; ln 29-35	6-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2009 (24.02.2009)	02 MAR 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

79532-8003.WO00
PDM/CDK

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PERKINS COIE LLP

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

DOCKETED TO CPI
☑ Deadline
☐ Follow up
☐ Previously
1/30/13 ☐ Abandoned
7/30/13 ☐ Transferred
☐ Dedocketed

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 20 JUN 2012
Applicant's or agent's file reference 795328003WO	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US2012/028620	International filing date *(day/month/year)* 09 March 2012
Applicant SCHARSCHMIDT, BRUCE	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:**

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, within **20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300
Facsimile No. 571-273-3201	Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328003WO	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2012/028620	International filing date *(day/month/year)* 09 March 2012	(Earliest) Priority Date *(day/month/year)* 30 September 2011
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the language, the international search was carried out on the basis of:

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

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

   b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (see Box No. II).

3. ☐ Unity of invention is lacking (see Box No. III).

4. With regard to the title,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

   a. the figure of the drawings to be published with the abstract is Figure No. 2

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/028620

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61B 5/00; A61K 31/192; A61K 49/00; A61P 13/00 (2012.01)
USPC - 424/9.2; 514/568; 600/322, 341

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pathase, Google Patent, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0008859 A1 (SCHARSCHMIDT) 14 January 2010 (14.01.2010) entire document	1-7, 9-12 --- 8
Y	ENNS et al., Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders, N Engl J Med 356; 22, 31 May 2007. entire document.	8
A	US 6,219,567 B1 (EGGERS et al) 17 April 2001 (17.04.2001) entire document	1-12
A	LEE et al., Phase 2 Comparison of A Novel Ammonia Scavenging Agent with Sodium Phenylbutyrate in Patients with Urea Cycle Disorders: Safety, Pharmacokinetics, and Ammonia Control. Mol. Genet Metab. 100(3) July 2010 entire document	1-12
A	LICHTER-KONECKI et al., Ammonia Control with Urea Cycle Disorders (UCDs); Phase 2 comparison of sodium phenylbutyrate and glycerol phynylbutyrate. Mol. Genet. Metab. 103 5 May 2011. entire document	1-12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 June 2012	20 JUN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

	Date of mailing *(day/month/year)* 20 JUN 2012
Applicant's or agent's file reference 79532803WO	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/028620	09 March 2012	30 September 2011

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2

Applicant SCHARSCHMIDT, BRUCE

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

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

2. FURTHER ACTION

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

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

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

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	04 June 2012	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Box No. I Basis of this opinion

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

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

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

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

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

a. (means)

☐ on paper

☐ in electronic form

b. (time)

☐ in the international application as filed

☐ together with the international application in electronic form

☐ subsequently to this Authority for the purposes of search

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

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

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

1. Statement

Novelty (N)	Claims	8	YES
	Claims	1-7, 9-12	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-7 and 9-12 lack novelty under PCT Article 33(2) as being anticipated by Scharschmidt et al. (hereafter Scharschmidt).

Regarding claim 1, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]);
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (if the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26. 1 umol/L), Para. [0201]).

Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0084]) comprising:

a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (if the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]);

Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]).

Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]).

Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]).

Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]).

Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]).

Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects is between 26 to 35 umol/L, Para. [0094]).

Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising:
c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and
e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Claim 8 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al.(hereafter Scharschmidt) in view of Ennis et al. (hereafter Ennis).

Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). It would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with lethal urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16).

Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

...

795328-8004.WO00
PDM/CDK

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

RECEIVED
PATENT DOCKETING
NOV 21 2012
PERKINS COIE LLP

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing (day/month/year): 20 NOV 2012

Applicant's or agent's file reference: 795328004WO0	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No.: PCT/US 12/54673	International filing date (day/month/year): 11 September 2012 (11.09.2012)
Applicant: SCHARSCHMIDT, BRUCE	

DOCKETED TO CPT
8/2/13
2/10/14
☐ Follow up
☐ Previously
☐ Abandoned
☐ Transferred
☐ Dedocketed

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328004WO0	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 12/54673	International filing date *(day/month/year)* 11 September 2012 (11.09.2012)	(Earliest) Priority Date *(day/month/year)* 20 April 2012 (20.04.2012)
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

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

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

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/54673

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/216; A61K 31/185
USPC - 514/533; 514/576; 514/532, 514/553; 554/220, 554/227
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/216; A61K 31/185 (2012.01)
USPC: 514/533; 514/576

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/532, 514/553; 554/220, 554/227 (search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, PatBase, Google Scholar: Plasma, PAA, PAGN, nitrogen retention, phenylacetic acid, phenylacetylglutamine, levels, NaPBA, NPH-100, nitrogen retention disorders, target range, dose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0022157 A1 (SCHARSCHMIDT) 26 January 2012 (26.01.2012); para [0021], [0069], [0097], [0106], [0116], [0118], [0160], [0173], [0174], [0181], [0297]	1-13
Y	MCGUIRE et al., Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis, HEPATOLOGY, June 2010, Vol. 51, pages 2077-2085; abstract; page 2079, col 2, para 3, page 2081, col 1, para 2;	1-13

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2012 (24.10.2012)	20 NOV 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 NOV 2012
Applicant's or agent's file reference 795328004WO0	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/54673	11 September 2012 (11.09.2012)	20 April 2012 (20.04.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 31/216; A61K 31/185 (2012.01)
USPC - 514/533; 514/576; 514/532, 514/553; 554/220, 554/227

Applicant SCHARSCHMIDT, BRUCE

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

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

2. **FURTHER ACTION**

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

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

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

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 October 2012 (24.10.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/54673

Box No. I Basis of this opinion

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

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

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

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

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

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

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

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/54673

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

1. Statement

Novelty (N)	Claims	1-13	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-13	NO
Industrial applicability (IA)	Claims	1-13	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-13 lack an inventive step under PCT Article 33(3) as being obvious over US 2012/0022157 A1 (Scharschmidt) in view of the article entitled, 'Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis' by McGuire et al. (hereinafter 'McGuire').

Regarding claim 1, Scharschmidt teaches a method of treating a nitrogen retention disorder in a subject (para [0173]) comprising:
(a) administering a first dosage of a PAA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]).
Scharschmidt however, fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (page 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 2, Scharschmidt teaches a method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug (para [0106], [0173]) comprising:
(a) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (b) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(d) administering a second dosage of the P AA prodrug based on the determination in (c) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
in light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

*****Continued in Supplemental Box*****

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 3, Scharschmidt teaches a method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject (para [0116], [0173]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the P AA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 4, Scharschmidt teaches a method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject (para [0116]) who has previously been administered a first dosage of a P AA prodrug (para [0106]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 5, Scharschmidt teaches a method of adjusting the dosage of a PAA prodrug (para [0021]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the P AA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

*****Continued in Next Supplemental Box*****

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/54673

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 6, Scharschmidt teaches a method of optimizing the therapeutic efficacy of a PAA prodrug in a subject (para [0297], [0173]) who has previously been administered a first dosage of a PAA prodrug (para [0106]) comprising:
(a) measuring PAGN levels (para [0174]), however fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (b) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(d) administering a second dosage of the P AA prodrug based on the determination in (c) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 7, the combination of Scharschmidt and McGuire makes obvious the method of claim 1, and Scharschmidt further teaches wherein the nitrogen retention disorder is selected from the group consisting of UCD (para [0097]).

Regarding claim 8, the combination of Scharschmidt and McGuire makes obvious the method of claim 3, and Scharschmidt further teaches wherein the disorder is a metabolic disorder (para [0046]).

Regarding claims 9-10, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 1, but fails to teach wherein the target range is 1 to 2.5 or wherein the target range is 1 to 2. It would have been obvious to an artisan of ordinary skill to determine the optimal target range for the plasma PAA:PAGN ratio for the subject being treated, based on routine experimentation, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 11, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 1, and Scharschmidt further teaches wherein measurement PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state (para [0160]), but fails to teach wherein the PAA levels are measured. It would have been obvious to one of ordinary skill in the art to further measure the PAA at the same time as the PAGN in order to maintain comparable results.

Regarding claim 12, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 11, and Scharschmidt further teaches wherein measurement of PAGN levels is carried out 48 hours to 1 week after the first dosage of PAA prodrug is administered (para [0160], 3 days), but fails to teach wherein the PAA levels are measured. It would have been obvious to one of ordinary skill in the art to further measure the PAA at the same time as the PAGN in order to maintain comparable results.

Regarding claim 13, the combination of Scharschmidt and McGuire makes obvious the method of claim 1, and Scharschmidt further teaches wherein the PAA prodrug is HPN-I00 (para [0118]).

Claims 1-13 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Docketed:
Amend Claims: 05/28/14
File Response: 09/21/14

REVIEWED
By Renee George at 3:07 pm, Apr 02, 2014

079532-8005.WO00
PDM/CDK

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

RECEIVED
PATENT DOCKETING

APR 0 2 2014

PERKINS COIE LLP

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day month year)* 28 MAR 2014
Applicant's or agent's file reference 795328005WO0	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US 13/71333	International filing date *(day month year)* 21 November 2013 (21.11.2013)
Applicant SCHARSCHMIDT, BRUCE	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4 **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis* 3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Lee W. Young PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328005WO0	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 13/71333	International filing date *(day/month/year)* 21 November 2013 (21.11.2013)	(Earliest) Priority Date *(day/month/year)* 21 November 2012 (21.11.2012)
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

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

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

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☐ the text is approved as submitted by the applicant.

   ☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☒ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/71333

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The present disclosure provides methods for treating hepatic encephalopathy (HE) and for optimizing and adjusting nitrogen scavenging drug dosage for subjects with HE, comprising administering a nitrogen scavenging drug at a dosage sufficient to maintain a fasting blood ammonia level at or below a specified threshold level which is 1.5 times the upper limit of normal for blood ammonia. The nitrogen scavenging drug administered in the method is a phenylacetic acid prodrug selected from HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/71333

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/9.1; 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), PubWest, FreePatentsOnline, Google Web search terms: hepatic encephalopathy blood plasma ammonia NH3 nitrogen phenylacetyl glutamine PAGN scavenging PAA phenylacetic acid prodrug sodium benzoate glyceryl tri phenylbutyrate (HPN-00) phenylbutyric acid PBA NaPBA measure

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0008859 A1 (Scharschmidt) 14 January 2010 (14.01.2010), para [0020], [0039], [0041], [0044], [0064], [0094], [0099], [0142], [0148], [0201], [0212], [0217]	9-12
Y		1-8
Y	Stauch et al., "Oral L-ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study" Journal of Hepatology, May 1998, Vol 28, Issue 5, Pages 856-864, [retrieved from internet: <URL: http://www.sciencedirect.com] pg 860, col 1, para 2- col 2, para 3; pg 862, col 1, para 3; pg 863, col 1, para 1	1-8
Y	Enns et al., "Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders", N Engl J Med., 31 May 2007 (31.05.2007), Vol. 356, pages 2282-2292, [retrieved from the Internet: <URL: http://www.nejm.org] abstract, para 1-4	5-6
A	US 2012/0220661 A1 (Lee) 30 August 2012 (30.08.2012), para [0034], [0090], [0094]	1-12
A	Lee et al., "Phase 2 comparison of a novel ammonia scavenging agent with sodium phenylbutyrate in patients with urea cycle disorders: Safety, pharmacokinetics and ammonia control", Molecular Genetics and Metabolism, July 2010, Vol 100, Issue 3, pages 221-228, [retrieved from the Internet: <URL: http://www.sciencedirect.com] pg 1-4	1-12
X, P	US 2013/0210914 A1 (Scharschmidt et al.) 15 August 2013 (15.08.2013), para [0010]-[0012], [0019], [0023]-[0031], [0038], [0042], [0057]	1-12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2014 (28.02.2014)	28 MAR 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* 28 MAR 2014

Applicant's or agent's file reference
795328005WO0

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 13/71333	21 November 2013 (21.11.2013)	21 November 2012 (21.11.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568

Applicant SCHARSCHMIDT, BRUCE

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

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

2. FURTHER ACTION

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

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

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

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	28 February 2014 (28.02.2014)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

Box No. I Basis of this opinion

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

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

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

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

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

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

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

5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

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

1. Statement

Novelty (N)	Claims	1-8	YES
	Claims	9-12	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	none	NO

2. Citations and explanations:

Claims 9-12 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0008859 A1 (Scharschmidt).

Regarding claim 9, Scharschmidt teaches a method (para [0039]) of treating hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) in a subject (para [0142], patient) in need thereof
comprising:
(a) determining a target urinary phenylacetyl glutamine (PAGN) output (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen);
(b) calculating an effective initial dosage of a PAA prodrug (para [0142], a method to determine an effective dosage of HPN-100 for a patient...monitoring the effect of an initial dosage of HPN-100) to achieve the target PAGN output based on a mean conversion of PAA prodrug to urinary PAGN of 52% to 63% (para [0148], determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60 percent to about 75 percent conversion of the PAA prodrug into urinary PAGN) and
(c) administering the effective initial dosage of PAA prodrug to the subject (para [0142], administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level in the patient).

Regarding claim 10, Scharschmidt teaches a method of claim 9, wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (para [0020], administered nitrogen scavenging drugs, including sodium phenylbutyrate (NaPBA) and glyceryl tri-[4-phenylbutyrate] (HPN-100)).

Regarding claim 11, Scharschmidt teaches a method of claim 9, further comprising a step of determining the upper limit of normal for blood ammonia for the subject (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level...can be a level of about 35 or about 40 micro mol/L; para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Regarding claim 12, Scharschmidt teaches a method of claim 9, wherein the upper limit of normal blood ammonia is 35 Lmol/L (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Claims 1-4, 7, and 8 lack an inventive step under PCT Article 33(3) as being obvious over Scharschmidt, in view of the article titled "Oral L -ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study" to Stauch et al. (hereinafter 'Stauch').

Regarding claim 1, Scharschmidt teaches a method (para [0039]) of treating hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) in a subject comprising:
(a) measuring a fasting blood ammonia level (para [0212], PK/PD modeling (a measurement) of ammonia in fasted and fed);
(b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L...; para [0094], plasma levels of ammonia are acceptable when they are at or below a level considered normal...the upper limit of normal for the subjects was between 26 and 35 micro mol/L); and
(c) administering a nitrogen scavenging drug to the subject (para [0041], determining and adjusting the dose of an ammonia scavenging drug to be administered to a patient with liver disease, including hepatic encephalopathy).
Scharschmidt does not specifically teach administering a nitrogen scavenging drug to the subject if the fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia.

Stauch teaches treatment of hepatic encephalopathy (HE) with OA (L-ornithine-L-aspartate) and further teaches OA decreases hyperammonemia (blood ammonia level) and improves HE (pg 862, col 1, para 3, confirms the beneficial effects already proven for OA-infusions in terms of the reduction in hyperammonemia and improvement in overt HE; pg 860, col 2, para 2, OA-treated patients with hepatic encephalopathy revealed a reduction in the fasting venous blood ammonia level (day 0: 87.5 +/- 28.8 micro mol/L; day 14: 52.2 +/- 27.8 micro mol/L).

-- Please see Supplemental Box for continuation of claim 1 --

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V. No 2: Citations and Explanations:
(Continued from Claim 1)

Stauch further teaches measuring the fasting blood ammonia level prior to and after administering OA to a subject (patient), and the pretreatment fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia (pg 860, col 1, para 2, pretreatment fasting blood ammonia concentrations (median) were similar in the placebo (84 micro mol/L) and OA groups (77 micro mol/L); pg 863, col 1, para 1, upper limit of normal (50 micro mol/L)).
To a person of ordinary skill in the art it would have been obvious to measure the fasting blood ammonia level prior to administering a drug to a subject (patient), and to check if it is greater than 1.5 times the upper limit of normal for blood ammonia as taught by Stauch when administering a nitrogen scavenging drug to a subject as taught by Scharschmidt. This is because both Scharschmidt (para [0201], patients with higher ammonia levels greater decreases in ammonia values following administration of HPN-100) and Stauch (pg 5, para 3) are directed toward the treatment of hepatic encephalopathy through lowering blood ammonia level.

Regarding claim 2, Scharschmidt in view of Stauch teach a method of claim 1 as discussed above. Scharschmidt further teaches wherein the subject has previously been administered a first dosage of a nitrogen scavenging drug (para [0044], method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate).

Regarding claim 3, Scharschmidt in view of Stauch teach a method of claim 2 as discussed above. Scharschmidt further teaches wherein the dosage of nitrogen scavenging drug administered in step (c) is greater than the first dosage (para [0083], plasma or blood level of ammonia is optionally also determined...If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug may need to be increased).

Regarding claim 4, Scharschmidt teaches a method (para [0039]) of optimizing the dosage of a nitrogen scavenging drug (para [0020], adjusting the schedule and dose of orally administered nitrogen scavenging drugs) for the treatment of hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) comprising:
(a) administering a first dosage of a nitrogen scavenging drug (para [0044], method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate);
(b) measuring a fasting blood ammonia level (para [0212], PK/PD modeling (a measurement) of ammonia in fasted and fed);
(c) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L...; para [0094], plasma levels of ammonia are acceptable when they are at or below a level considered normal...the upper limit of normal for the subjects was between 26 and 35 micro mol/L) to determine whether to increase the dosage of a nitrogen scavenging drug (para [0041], determining and adjusting the dose of an ammonia scavenging drug to be administered), wherein the dosage needs to be increased (para [0099], adjusting the initial dosage of the new drug as needed to provide an adjusted dosage based upon ammonia control); and
(d) administering a second dosage of the nitrogen scavenging drug based on the determination in (c) (para [0113], this method comprises adjusting the amount of the prodrug and administering an adjusted amount of the prodrug).

Scharschmidt does not specifically teach dosage needs to be increased if the fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia.

Stauch teaches treatment of hepatic encephalopathy (HE) with OA (L-ornithine-L-aspartate) and further teaches OA decreases hyperammonemia (blood ammonia level) and improves HE (pg 862, col 1, para 3, confirms the beneficial effects already proven for OA-infusions in terms of the reduction in hyperammonemia and improvement in overt HE; pg 860, col 2, para 2, OA-treated patients with hepatic encephalopathy revealed a reduction in the fasting venous blood ammonia level (day 0: 87.5 +/- 28.8 micro mol/L; day 14: 52.2 +/- 27.8 micro mol/L).

Stauch further teaches measuring fasting blood ammonia level prior to and after administering OA to a subject (patient), and the pretreatment fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia (pg 860, col 1, para 2, pretreatment fasting blood ammonia concentrations (median) were similar in the placebo (84 micro mol/L) and OA groups (77 micro mol/L); pg 863, col 1, para 1, upper limit of normal (50 micro mol/L)).
To a person of ordinary skill in the art it would have been obvious to measure the fasting blood ammonia level prior to administering a drug to a subject (patient), and to check if it is greater than 1.5 times the upper limit of normal for blood ammonia as taught by Stauch when administering a nitrogen scavenging drug to a subject as taught by Scharschmidt. This is because both Scharschmidt (para [0201], patients with higher ammonia levels greater decreases in ammonia values following administration of HPN-100) and Stauch (pg 5, para 3) are directed toward the treatment of hepatic encephalopathy through lowering blood ammonia level.

Regarding claim 7, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above. Scharschmidt further teaches comprising a step of determining the upper limit of normal for blood ammonia for the subject (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level...can be a level of about 35 or about 40 micro mol/L; para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Regarding claim 8, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above. Scharschmidt further teaches wherein the upper limit of normal blood ammonia is 35 .mol/L (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

-- Please see Next Supplemental Box --

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Previous Supplemental Box:

Claims 5-6 lack an inventive step under PCT Article 33(3) as being obvious over Scharschmidt, in view of Stauch, further in view of the article titled "Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders" to Enns et al. (hereinafter 'Enns') .

Regarding claim 5, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above.
Scharschmidt further teaches wherein the nitrogen scavenging drug is a PAA prodrug (para [0217], the preclinical handling of ammonia scavenging drugs, 600 mg/kg of either radio labeled sodium PBA or radio labeled HPN-100 was administered as a single dose to 3 cynomolgous monkeys. These monkeys were chosen because, like humans (and unlike most other species), they metabolize PAA to PAGN and thus provide a useful model for testing prodrugs of PAA).

Scharschmidt does not specifically teaches the nitrogen scavenging drug is sodium benzoate.

Enns teaches treating urea cycle disorder (relates to hepatic encephalopathy) with phenylacetate and benzoate and further teaches the use of sodium benzoate to treat patients with ammonia disorders (abstract, para 1-4, sodium benzoate therapy in patients). To a person of ordinary skill in the art it would have been obvious to use the therapeutic drug sodium benzoate as taught by Enns with the method of Scharschmidt in view of Stauch. The motivation would be to lower plasma (blood) ammonia levels and improve the survival of patients with lethal urea-cycle enzyme defects (Enns) (abstract, para 1-4, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects).

Regarding claim 6, Scharschmidt in view of Stauch, further in view of Enns teach a method of claim 5, wherein Scharschmidt further teaches the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (para [0020], administered nitrogen scavenging drugs, including sodium phenylbutyrate (NaPBA) and glyceryl tri-[4-phenylbutyrate] (HPN-100)).

Claims 1-12 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Electronic Acknowledgement Receipt	
EFS ID:	20748225
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Lara J. Dueppen/Deborah Muench
Filer Authorized By:	Lara J. Dueppen
Attorney Docket Number:	079532-8003.US03
Receipt Date:	19-NOV-2014
Filing Date:	22-FEB-2013
Time Stamp:	22:02:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	2014-11-19_IDS-Supplemental_Transmittal_795328003US03.pdf	83900 b765f96f2dde325a4d342d29cc5842097c484eb2	no	3
Warnings:					
Information:					

2	Information Disclosure Statement (IDS) Form (SB08)	2014-11-19_IDS-Supplemental_Form_PTO-1449_795328003US03.pdf	133493 b3b40e25b856bb830930aab805139e46f89aa6a2	no	3
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Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	WO2007005633.PDF	958722 baf2b4dfd4478077b257c00235c9af6ed5bd960a	no	19
Warnings:					
Information:					
4	Foreign Reference	WO2009087474A2.PDF	3777890 9cca4e5b3be5a80b0360dec55d494634306d1c9c	no	67
Warnings:					
Information:					
5	Foreign Reference	WO2012028620.PDF	2882713 c0b0a285b0bd13be60dca656aa0e60019d11b934	no	48
Warnings:					
Information:					
6	Non Patent Literature	Batshaw_M_1981_Pediatrics_68_290-297.PDF	1417261 7b232b6720fe9fcece6df359d2b6c08cf0e1717d	no	10
Warnings:					
Information:					
7	Non Patent Literature	Brahe_C_2005_EurJHumGenet_13_256-259.PDF	115135 8b0fab1febdd66cad229d931f42430338d393cb9	no	4
Warnings:					
Information:					
8	Non Patent Literature	Brunetti-Pierri_2011_HumMolGenet_20_631-640.PDF	262898 2b9223a07189ae82aedb9e49accea256a84692fc	no	10
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Information:					
9	Non Patent Literature	Chung_YL_ClinCancerRes_2000_6_1452-1458.PDF	750618 2ff26b5a3a6db9969211cd8c2f43dd2e14910501	no	8
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Information:					
10	Non Patent Literature	Cudkowicz_M_2009_AmyotrophicLateralSclerosis_10_99-1106.PDF	123014 c38180d9a9bd4706d6ca6dce4c617a9021961a49	no	8

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11	Non Patent Literature	Enns_GM_2007_NEngJMed_356_2282-2292.PDF	222044 8c38e91725e2fb538cc51886287f207545f9b762	no	11
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Information:					
12	Non Patent Literature	Gropman_A_2010_MolGenetMetab_100_S20-S30.PDF	1566360 f2ef8b75ca076e6672115c6db4239569c794dc86	no	11
Warnings:					
Information:					
13	Non Patent Literature	Hines_P_2008_PediatrBloodCancer_50_357-359.PDF	106950 0f5c83da84f176268df5f58ec38f5743c6538b47	no	3
Warnings:					
Information:					
14	Non Patent Literature	Hogarth_P_2007_MovDisorders_22_1962-1964.PDF	57861 7c1d94ea74bd8ac5dad1ac1de21d3be6fc801908	no	3
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Information:					
15	Non Patent Literature	Huang_H_2012_Hepatology_56_248-258.PDF	1455550 ffe291036302ede5513c387082ac265daa701689	no	11
Warnings:					
Information:					
16	Non Patent Literature	HyperionPressRes_10232007.PDF	87009 4ebd52e262a117abe39c5b2bc90109b59740be2d	no	1
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Information:					
17	Non Patent Literature	Mercuri_E_2004_NeuromusculDisord_14_130-135.PDF	214339 0bc7927cef71f03b15712509842fb202317242bc	no	6
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Information:					
18	Non Patent Literature	Mokhtarani_M_2012_MolGenetMetab_Abstract_105_342-343.PDF	61319 9cfbc082c1d3e87683b45d47d0eecd6bc505fcd6	no	2
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19	Non Patent Literature	Diaz_SIMD_ph_III_final_abstract.pdf	83801 dfe49360f2a50dea5582fca8302eb9e4696d5652	no	2

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20	Non Patent Literature	Moldave_K_1957_JBiolChem_229_463-476.pdf	2052268 8d26e0a79ef3fa41094e561833d01a5e5966bc16	no	15
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Information:					
21	Non Patent Literature	Monteleone_J_2012_MolGenetMetab_105_343.PDF	242644 4c69fa985e537a906beb0d1dc79b0ffa2404851e	no	2
Warnings:					
Information:					
22	Non Patent Literature	Ong_J_2003_AmJMed_114_188-193.PDF	113922 1cf4dd4f85d5bc2a8902ccabdc1e7031539bc3f8	no	6
Warnings:					
Information:					
23	Non Patent Literature	Perrine_S_2008_PediatrAnn_37-339-346.pdf	1247020 b5ec8c0239677236665acd8e1afb6989e7be57ec	no	10
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Information:					
24	Non Patent Literature	Ryu_H_2005_JNeurochem_93_1087-1098.PDF	819106 f46c5411bbf08d35b97164d59b415d0f9b75f2de	no	12
Warnings:					
Information:					
25	Non Patent Literature	Stauch_S_1998_JHepatology_28_856-864.PDF	296302 bcff3f692e8a5aafbded52152a4b2060a766e604	no	9
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Information:					
26	Non Patent Literature	Xie_G_2012_Gastroenterology_142_918-927.PDF	6518066 3257c9a1c2831745b17a551bed9a2f6a2512081a	no	16
Warnings:					
Information:					
27	Other Reference-Patent/App/Search documents	2011-11-02_eESR-EP09739263.PDF	497036 470bc7c9f91e5a73ccffcfe2cdd0ef670989d6cd	no	6
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Information:					
28	Other Reference-Patent/App/Search documents	2009-12-30_ISR-EPandWO.PDF	1249139 4eb380a535bfb8943ff342a7c27b292b139a12ad	no	13

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29	Other Reference-Patent/App/Search documents	2011-10-28_GB_Examination_Report-GB1013468-2.PDF	257798 c985e27cc2abd0ad7ebf08418b52277508a49f87	no	2
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30	Other Reference-Patent/App/Search documents	2014-04-10_IPRP_Ch_I-PCTUS2012028620.PDF	1648474 99fc5ffdcf3dc82c792003669d6ead0e30b7deaa	no	7
Warnings:					
Information:					
31	Other Reference-Patent/App/Search documents	2013-09-04_IPRP_Ch_II-PCTUS2012028620.PDF	458238 24b2b74ecc36a476dd2cfe125acf3a958ae18fca	no	6
Warnings:					
Information:					
32	Other Reference-Patent/App/Search documents	2009-03-02_ISRandWO.PDF	568842 4464dfcb205cfed0e99babd63236056be8926404	no	9
Warnings:					
Information:					
33	Other Reference-Patent/App/Search documents	2012-06-20_ISRandWO.pdf	6733198 0b9bb6f10def7c9daa55ae6d97dc6e935153e0dd	no	8
Warnings:					
Information:					
34	Other Reference-Patent/App/Search documents	2012-11-20_ISRandWO.pdf	10693349 55b8bd529db22fd272ab401381cc766c8b4e2954	no	8
Warnings:					
Information:					
35	Other Reference-Patent/App/Search documents	2014-03-28_ISRandWO.pdf	17460908 17b517e60a6d0eb90abccee544ebd21970139cd1	no	9
Warnings:					
Information:					
		Total Files Size (in bytes):	65217187		

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: BRUCE SCHARSCHMIDT ET AL.	CONF. NO: 7929
APPLICATION NO.: 13/775,000	ART UNIT: 1736
FILED: FEBRUARY 22, 2013	
FOR: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Supplemental Information Disclosure Statement Within Three Months of Application Filing or Before First Action – 37 C.F.R. § 1.97(b)

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. Timing of Submission

This information disclosure is being filed within three months of the filing date of this application or date of entry into the national stage of an international application or before the mailing date of a first Office action on the merits, whichever occurs last [37 C.F.R. § 1.97(b)]. The references listed on the enclosed Form PTO-1449 (modified) may be material to the examination of this application; the Examiner is requested to make them of record in the application.

2. Cited Information

☒ Copies of the following references are enclosed:

☒ All cited references
☐ References marked by asterisks
☐ The following:

☐ Copies of the following references can be found in parent U.S. Application No. <>:

- ☐ All cited references
- ☐ All references
- ☐ The following:

☒ This application was filed after 30 June 2003 and no copies of U.S. patents nor published applications are enclosed (See Notice of Deputy Commissioner Kunin on 11 July 2003).

☐ The following references are not in English. For each such reference, the undersigned has enclosed (i) a translation of the reference; (ii) a copy of a communication from a foreign patent office or International Searching Authority citing the reference, (iii) a copy of a reference which appears to be an English-language counterpart, or (iv) an English-language abstract for the reference prepared by a third party. Applicant has not verified that the translation, English-language counterpart or third-party abstract is an accurate representation of the teachings of the non-English reference, though, and reserves the right to demonstrate otherwise.

- ☐ All cited references
- ☐ References marked by ampersands
- ☐ The following:

3. Effect of Information Disclosure Statement (37 C.F.R. § 1.97(h))

This Information Disclosure Statement is not to be construed as a representation that: (i) a search has been made; (ii) additional information material to the examination of this application does not exist; (iii) the information, protocols, results and the like reported by third parties are accurate or enabling; or (iv) the cited information is, or is considered to be, material to patentability. In addition, applicant does not admit that any enclosed item of information constitutes prior art to the subject invention and specifically reserves the right to demonstrate that any such reference is not prior art.

4. Fee Payment

No fees are believed due because this Information Disclosure Statement is being filed before the mailing date of the first Office Action.

☐ Applicant further submits that no fee is due in light of the following certification under 37 C.F.R. § 1.97(e) (check only one):

- ☐ In accordance with 37 C.F.R. § 1.97(e)(1), the undersigned hereby states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart

foreign application not more than three months prior to the filing of this statement; or

☐ In accordance with 37 C.F.R. § 1.97(e)(2), the undersigned hereby states that no item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart foreign application, or, to the knowledge of the person signing the certification after making reasonable inquiry, was known to any individual designated in 37 C.F.R. § 1.56(c), more than three months prior to the filing of this statement.

However, should the Commissioner determine that fees are due in order for this Information Disclosure Statement to be considered, the Commissioner is hereby authorized to charge such fees to Deposit Account No. 50-2586.

5. Patent Term Adjustment (37 C.F.R. § 1.704(d))

☐ The undersigned states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart application and that this communication was not received by any individual designated in 37 C.F.R. § 1.56(c) more than thirty days prior to the filing of this statement. 37 C.F.R. § 1.704(d).

Respectfully submitted,
Perkins Coie LLP

Date: November 19, 2014

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03	7929

34055 7590 01/09/2015

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POST OFFICE BOX 1247
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EXAMINER	
RAO, SAVITHA M	

ART UNIT	PAPER NUMBER
1621	

NOTIFICATION DATE	DELIVERY MODE
01/09/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentprocurement@perkinscoie.com

Office Action Summary	Application No. 13/775,000	Applicant(s) SCHARSCHMIDT ET AL.	
	Examiner SAVITHA RAO	Art Unit 1621	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02/22/2013*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-11* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-11* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *04/18/2013 and 11/19/2014* .

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Claims 1-11 are pending and are under consideration in the instant office action.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 04/18/2013 and 11/19/2014 complies with the provisions of 37 CFR 1.97, 1.98 and MPEP § 609. Accordingly, it has been placed in the application file and the information therein has been considered as to the merits. See attached copy of the PTO-1449.

Priority

This application is a divisional of application 13/147,317 dated 03/19/2012 (granted as a patent number 8404215) which claims priority under 35 U.S.C 119 (e) from provisional application serial No. 61/564668 filed 11/29/2011 and provisional application no 61/542100 filed on 09/30/2011.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent

and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 12-11 are rejected on the ground of nonstatutory double patenting over claim 1-6 and 8-11 of U. S. Patent No 8,404,215 ('215) since the claims, if allowed, would improperly extend the "right to exclude" already granted in the patent.

Claim 1-23, 33-48, 59-72 and 80-88 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1-3, 4-6, 8-13, 15-17, 18-20, 27-33, 43-48, 50-55, 57-63 and 64-69 of U.S. Patent No. 7,838,532 (co-pending '532) further in view of Sinclair et al.(US 2006/0276416)

An obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but an examined application claim not is patentably distinct from the reference claim(s) because the examined claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985).

The subject matter claimed in the instant application is fully disclosed in the patent and is covered by the patent since the patent and the application are claiming common subject matter.

Claim 1 of '215 states as follows:

1. A method for adjusting the dosage of a nitrogen scavenging drug in a subject who has previously been administered an initial dosage of the nitrogen scavenging drug, comprising:

a) measuring a fasting blood ammonia level for the subject;

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level; and

c) administering an adjusted dosage of the nitrogen scavenging drug, wherein the adjusted dosage is greater than the initial dosage if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level.

Claim 3 of '215 states as follows

3. A method of treating a subject with a nitrogen retention disorder who has previously been administered an initial dosage of a nitrogen scavenging drug comprising:
a) measuring a fasting blood ammonia level for the subject;
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level; and
c) administering an adjusted dosage of the nitrogen scavenging drug that is greater than the initial dosage if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level.

Dependent claims recite the nitrogen retention disorder to be urea cycle disorder ('215 claim 4) and the nitrogen scavenging drug to be glyceryl tri-(4-phenylbutyrate) (reference claim 6) which is instantly claimed. The other limitations instantly claimed in claims 1-11 are recited in the claims of parent patent '215.

Although the conflicting claims are not identical, they are not patentably distinct from each other. The instant claims are rendered prima facia obvious to a person of ordinary skill in the art to utilize the specific agent instantly claimed which is taught in claim 6 of '215 in the methods of claim 1 and 3 of '215 where in the nitrogen retention disorder is an urea cycle disorder. It is also noted that the steps in following the instant method is the same as that claimed in '215.

Conclusion

Claims 1-11 are rejected. No claims are allowed

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAVITHA RAO whose telephone number is (571)270-5315. The examiner can normally be reached on Mon-Fri 7 am to 4 pm..

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Melanie McCormick can be reached at 571-272-8037. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SAVITHA RAO/
Primary Examiner, Art Unit 1621

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
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S3	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/15 13:46
S4	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/11/15 13:56
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S9	8	S7 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:08
S10	2	S9 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:08
S11	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:12
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S64	2	S63 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S65	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR;	OR	OFF	2012/12/20 16:43

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S75	13	S74 and PAA	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2014/12/18 12:08
S76	11	"glyceryl tri-[4-phenylbutyrate]"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/12/18 12:09
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S78	14	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/12/18 12:12
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EAST Search History (Interference)

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12/21/2014 10:32:20 PM
C:\Users\srao3\Documents\EAST\Workspaces\13775000.wsp

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13775000	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SAVITHA RAO	1621

CPC- SEARCHED		
Symbol	Date	Examiner

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
eaST search (See attached)	12/21/2014	SR
Inventor search in EAST and PALM	12/21/2014	SR
Reviewed STN searches from the Parent application, further NPL search in Google	12/21/2014	SR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

	/SAVITHA RAO/ Primary Examiner.Art Unit 1621

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Form PTO-1449 (Modified)

(Use several sheets if necessary)

Sheet	1	of	3

COMPLETE IF KNOWN	
Application Number	13/775,000
Confirmation Number	7929
Filing Date	February 22, 2013
First Named Inventor	SCHARSCHMIDT, Bruce
Group Art Unit	1736
Examiner Name	
Attorney Docket No.	79532.8003.US03

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	U.S. Patent or Application NUMBER	Kind Code (if known)	Name of Patentee or Inventor of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	6,219,567		EGGERS	4/17/2001	
	A2	8,642,012		SCHARSCHMIDT	2/4/2014	
	A3	2010/0008859		SCHARSCHMIDT	1/14/2010	
	A4	2012/0022157		SCHARSCHMIDT		
	A5	2012/0220661		LEE		
	A6	2013/0210914		SCHARSCHMIDT		

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.	Foreign Patent or Application Office	NUMBER	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	B1	WO	2007/005633					
	B2	WO	2009/087474		Akthelia Pharmaceuticals	7/16/2009		
	B3	WO	2012/028620					

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
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SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Form PTO-1449 (Modified)
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Sheet	2	of	3

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Filing Date	February 22, 2013
First Named Inventor	SCHARSCHMIDT, Bruce
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Examiner Name	
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	A2	2006/0135612	A1	FERRANTE	06/22/2006	
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	B2	WO	2006/056794		UCL BUSINESS PLC	06/01/2006		
	B3	WO	2009/087474		AKTHELIA PHARMACEUTICALS	07/16/2009		
	B4	WO	2009/134460	A1	HYPERION THERAPEUTICS	11/05/2009		
	B5	WO	2010/0250303	A1	HYPERION THERAPEUTICS	03/04/2010		

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	C51	LEE, B. et al. (September 2008). "Preliminary Data on Adult Patients with Urea Cycle Disorders (UCD) in An Open-Label, Switch-Over, Dose Escalation Study Comparing A New Ammonia Scavenger, Glyceryl Tri (4-Phenylbutyrate) [HPN-1 00], to BUPHENYL® (Sodium Phenylbutyrate [PBA]," presented at SSIEM 2008, Lisbon, Portugal, Poster, one page.	
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EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

Form PTO-1449 (Modified)
(Use several sheets if necessary)

Sheet	8	of	10

COMPLETE IF KNOWN	
Application Number	13/775,000
Confirmation Number	7929
Filing Date	2013-02-22
First Named Inventor	Bruce SCHARSCHMIDT
Group Art Unit	1736
Examiner Name	To be assigned
Attorney Docket No.	79532.8003.US03

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
	C58	MANSOUR, A. et al. (October 1997). "Abdominal Operations in Patients with Cirrhosis: Still A Major Surgical Challenge," Surqerv 122(4):730-735. (Abstract Only.)	
	C59	MASETRI, N.E. et al. (August 1992). "Plasma Glutamine Concentration: A Guide in the Management of Urea Cycle Disorders," J. Pediatr. 121 (2):259-261.	
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	C64	MCGUIRE, B. et al. (April 2008). "Pharmacokeinetic (PK) Safety Study of Sodium Phenylacetate and Sodium Benzoate Administered to Subjects with Hepatic Impairment," abstract of The13th International Symposium, Abano (Padova), Italy, April 28-May 1,2008, two pages.	
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				Filing Date	2013-02-22
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				Group Art Unit	1736
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Sheet	9	of	10	Attorney Docket No.	79532.8003.US03

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS			
Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T
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	C69	RUDMAN, D., et al., "Maximal Rates of Excretion and Synthesis of Urea in Normal and Cirrhotic Subjects," J. Clin. Invest. (1973) 52:2241-2249.	
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EXAMINER /Savitha Rao/	DATE CONSIDERED 12/18/2014

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

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BIB DATA SHEET

CONFIRMATION NO. 7929

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
13/775,000	02/22/2013 RULE	424	1621	079532-8003.US03

APPLICANTS
HYPERION THERAPEUTICS, INC., South San Francisco, CA

INVENTORS
Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

**** CONTINUING DATA **********************
This application is a DIV of 13/417,137 03/09/2012 PAT 8404215 *
which claims benefit of 61/542,100 09/30/2011
and claims benefit of 61/564,668 11/29/2011
(*)Data provided by applicant is not consistent with PTO records.

**** FOREIGN APPLICATIONS **********************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
03/15/2013

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /SAVITHA M RAO/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CA	3	11	3

ADDRESS
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POST OFFICE BOX 1247
SEATTLE, WA 98111-1247
UNITED STATES

TITLE
METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

FILING FEE RECEIVED		
828	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **SCHARSCHMIDT, Bruce, et al.** **Serial No.:** 13/775,000 **Filed:** February 22, 2013 **For: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS**		**Examiner:** RAO, Savitha M. **Group Art Unit:** 1621 **Docket No.:** 079532.8003.US03 I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being deposited with the U.S. Patent and Trademark Office this 11th day of May 2015 via EFS-Web Electronic Filing. /Colleen Kirchner/ Colleen Kirchner

RESPONSE TO NON-FINAL OFFICE ACTION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The following is in response to the Non-Final Office Action mailed January 9, 2015 for the above-identified application.

Pending Claims begin on page 2.

Remarks begin on page 5.

Conclusion begins on page 6.

PENDING CLAIMS

1. (original) A method for adjusting the dosage of glyceryl tri-[4-phenylbutyrate] in a subject being treated for a urea cycle disorder who has previously been administered an initial dosage of glyceryl tri-[4-phenylbutyrate], the method comprising:

(a) measuring a fasting plasma ammonia level for the subject;

(b) comparing the fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate], wherein the adjusted dosage is greater than the initial dosage if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

2. (original) A method of treating a subject with a urea cycle disorder who has previously been administered an initial dosage of glyceryl tri-[4-phenylbutyrate], the method comprising:

(a) measuring a fasting plasma ammonia level for the subject;

(b) comparing the fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate] that is greater than the initial dosage if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

3. (original) A method of administering glyceryl tri-[4-phenylbutyrate] to a subject having a urea cycle disorder, the method comprising:

(a) measuring a first fasting plasma ammonia level for the subject;

(b) comparing the first fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(c) administering an initial dosage of glyceryl tri-[4-phenylbutyrate] to the subject if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

4. (original) The method of claim 1 or 2, wherein administering the adjusted dosage of glyceryl tri-[4-phenylbutyrate] produces a normal average daily ammonia level in the subject.

5. (original) The method of claim 1 or 2, further comprising repeating steps (a) to (c) until the subject exhibits a fasting plasma ammonia level at or below half the upper limit of normal for plasma ammonia level.

6. (original) The method of claim 3, further comprising:

(d) measuring a second fasting plasma ammonia level for the subject;

(e) comparing the second fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and

(f) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate] that is greater than the initial dosage if the second fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

7. (original) The method of any of claims 1-3, wherein the upper limit of normal for plasma ammonia level is 35 μmol/L.

8. (original) The method of any of claims 1-3, wherein the upper limit of normal is specific to the laboratory in which the fasting plasma ammonia level is measured.

9. (original) The method of any of claims 1-3, further comprising the step of determining an upper limit of normal for plasma ammonia level for the subject prior to step (b).

10. (original) The method of claim 1 or 2, wherein the adjusted dosage is calculated by:

(i) measuring urinary phenylacetyl glutamine (PAGN) output; and

(ii) calculating an effective adjusted dosage of glyceryl tri-[4-phenylbutyrate] based on the urinary PAGN output, wherein the effective adjusted dosage is calculated based on a mean conversion of glyceryl tri-[4-phenylbutyrate] to urinary PAGN of 60 to 75%.

11. (original) The method of claim 3, wherein the initial dosage is calculated by:

(i) determining a target urinary phenylacetyl glutamine (PAGN) output; and

(ii) calculating an effective initial dosage of glyceryl tri-[4-phenylbutyrate] based on a mean conversion of glyceryl tri-[4-phenylbutyrate] to urinary PAGN of 60 to 75%.

REMARKS

Claims 1-11 are pending in the present application and stand rejected.

Double patenting

The Office Action rejects claims 1-11 on the ground of nonstatutory double patenting over claims 1-6 and 8-11 of U.S. Patent No. 8,404,215 ("the '215 Patent"). Applicants have submitted herewith a terminal disclaimer over the '215 Patent.

The Office Action also includes a double patenting rejection based on U.S. Patent No. 7,838,532 in view of U.S. Patent Publ. No. 2006/0276416. In a telephone conversation on January 13, 2015, the Examiner indicated that this rejection was in error.

CONCLUSION

In view of the foregoing, it is submitted that the present claims are in condition for allowance. Accordingly, Applicant respectfully requests that a Notice of Allowance be issued. If Applicant can do anything more to expedite this application, Applicant requests that the Examiner contact the undersigned at (415) 344-7105.

Respectfully submitted,
Perkins Coie LLP

Date: May 11, 2015

/Patrick D. Morris/
Patrick D. Morris, Ph.D.
Registration No. 53,351

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Perkins Coie LLP
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **SCHARSCHMIDT, Bruce, et al.** **Serial No.:** 13/775,000 **Filed:** February 22, 2013 **For: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS**		**Examiner:** RAO, Savitha M. **Group Art Unit:** 1621 **Docket No.:** 079532.8003.US03 I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being deposited with the U.S. Patent and Trademark Office this 11th day of May 2015 via EFS-Web Electronic Filing. /Colleen Kirchner/ Colleen Kirchner

PETITION FOR A ONE-MONTH EXTENSION OF TIME

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant petitions for a one-month extension of time in which to respond to the Non-Final Office Action mailed January 9, 2015, extending the period for response to May 9, 2015. Payment of the one-month extension fee of $100 (Small Entity) is being charged to Deposit Account No. 50-2586.

Respectfully submitted,

Dated: May 11, 2015

PERKINS COIE LLP

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Patent - LA
P.O. Box 1208
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Phone: (310) 788-9900
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By: /Patrick D. Morris/
Patrick D. Morris
Reg. No. 53,351

PTO/AIA/26 (04-14)
Approved for use through 07/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) 079532-8003.US03

In re Application of: Bruce SCHARSCHMIDT et al.

Application No.: 13/775,000

Filed: February 22, 2013

For: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

The applicant, HYPERION THERAPEUTICS, INC., owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. 8,404,215 as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

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is held unenforceable;
is found invalid by a court of competent jurisdiction;
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Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 53,351

/Patrick D. Morris/ — Signature
May 11, 2015 — Date

Patrick D. Morris — Typed or printed name

Attorney of record — Title
(415) 344-7105 — Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) included.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	13775000
Filing Date:	22-Feb-2013
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Yingli Wang/Colleen Kirchner
Attorney Docket Number:	079532-8003.US03

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	2251	1	100	100
Miscellaneous:				
Statutory or Terminal Disclaimer	1814	1	160	160
		Total in USD ($)		**260**

Electronic Acknowledgement Receipt	
EFS ID:	22311072
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Yingli Wang/Colleen Kirchner
Filer Authorized By:	Yingli Wang
Attorney Docket Number:	079532-8003.US03
Receipt Date:	11-MAY-2015
Filing Date:	22-FEB-2013
Time Stamp:	16:37:48
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment was successfully received in RAM	$260
RAM confirmation Number	3692
Deposit Account	502586
Authorized User	KIRCHNER, COLLEEN
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

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Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Response.pdf	101991 d8bc664d659fb018cf675d95a97a79913d60e24c	yes	6

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	6

Warnings:

Information:

2	Extension of Time	Extension.pdf	89980 df21e4ed03ed7c9d887bfa8824db87ab7e416e95	no	1

Warnings:

Information:

3	Terminal Disclaimer Filed	Disclaimer.pdf	161128 ea314c802adf208f9f4187c7962c261550fc3e64	no	2

Warnings:

Information:

4	Fee Worksheet (SB06)	fee-info.pdf	32935 f44fd5b64f49e918ee309edeb1e6021e7c568922	no	2

Warnings:

Information:

Total Files Size (in bytes):	386034

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/775,000	Filing Date 02/22/2013	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	05/11/2015	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 20	Minus	** 20	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***3	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	**0**

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/PARTHENIA D. MERRILL/

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

■ Practitioners associated with Customer Number: 101325

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

■ The address associated with Customer Number: 101325

OR

☐

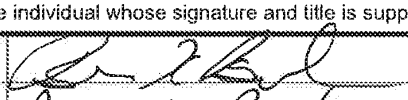
Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address: Horizon Therapeutics, Inc.
533 Bryant, Suite #6
Palo Alto, CA 94301

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	5/1/15
Name	Brian K. Beeler	Telephone	847-502-5250
Title	Senior VP, Legal		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office;U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of1995, no persons arerequired to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: HORIZON THERAPEUTICS, INC.

Application No./Patent No.: As set forth on the attached Schedule A Filed/Issue Date: As set forth on the attached Schedule A

Titled: _____

HORIZON THERAPEUTICS, INC., a Delaware Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

   ☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

   ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

   Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel See Schedule A, Frame See Schedule A, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

   The document was recorded in the United States Patent and Trademark Office at

   Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

   The document was recorded in the United States Patent and Trademark Office at

   Reel _____, Frame _____, or for which a copy thereof is attached.

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at

Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Dennis A. Bennett/	May 15, 2015
Signature	Date
Dennis A. Bennett	Attorney of Record, Reg No. 34547
Printed or Typed Name	Title or Registration Number

Schedule A

Docket No.	Application No.	Application Date	Reel/Frame No.	Recordation Date
079532-8001.US01	12/350,111	2009-01-07	022305 / 0387 025031 / 0014 028014 / 0894 035638 / 0305	02/24/2009 09/22/2010 04/09/2012 05/14/2015
079532-8003.US02	13/417,137	2012-03-09	028014 / 0894 035638 / 0305	04/09/2012 05/14/2015
079532-8003.US03	13/775,000	2013-02-22	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8004.US01	13/610,580	2012-09-11	029337 / 0054 035638 / 0305	11/21/2012 05/14/2015
079532-8005.US02	14/086,870	2013-11-21	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US00	61/890,827	2013-10-14	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US01	62/044,168	2014-08-29	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US02	14/514,334	2014-10-14	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015

Electronic Acknowledgement Receipt	
EFS ID:	22363965
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Dennis A. Bennett/Ronnie Almira
Filer Authorized By:	Dennis A. Bennett
Attorney Docket Number:	079532-8003.US03
Receipt Date:	15-MAY-2015
Filing Date:	22-FEB-2013
Time Stamp:	17:03:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	HorizonTherapeutics-POA_Assignee.pdf	96506 cb08b2aa2de030cfca8e0ff6ce3b34f18d522e9f	no	1
Warnings:					
Information:					

2	Assignee showing of ownership per 37 CFR 3.73	HOR_373-Statment_Schedule_A.pdf	157428 6c05c96d65f079637c44f6e854cbea479726c476	no	3

Warnings:	
Information:	
Total Files Size (in bytes):	253934

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Doc Code: N572

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03

CONFIRMATION NO. 7929

OC000000075263036

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

Cc: GLOBAL PATENT GROUP - HOR
1005 NORTH WARSON ROAD
SUITE 404
SAINT LOUIS, MO 63132

Date Mailed: 05/19/2015

DENIAL OF REQUEST FOR POWER OF ATTORNEY

The request for Power of Attorney filed 05/15/2015 is acknowledged. However, the request cannot be granted at this time for the reason stated below.

- ☐ The Power of Attorney you provided did not comply with the new Power of Attorney rules that became effective on June 25, 2004. See 37 CFR 1.32.
- ☐ The revocation is not signed by the applicant, the assignee of the entire interest, or one particular principal attorney having the authority to revoke.
- ☐ The Power of Attorney is from an assignee and the Certificate required by 37 CFR 3.73(c) has not been received.
- ☐ The person signing for the assignee has omitted their empowerment to sign on behalf of the assignee.
- ☐ The inventor(s) is without authority to appoint attorneys since the assignee has intervened as provided by 37 CFR 3.71.
- ☐ The signature(s) of _____ , a co-inventor in this application, has been omitted. The Power of Attorney will be entered upon receipt of confirmation signed by said co-inventor(s).
- ☐ The person(s) appointed in the Power of Attorney is not registered to practice before the U.S. Patent and Trademark Office.
- ☐ Only one Customer Number can be designated for the Power of Attorney in an application. The Customer Number that was captured is the first Customer Number provided on the Power of Attorney document.
- ☐ A request under 37 CFR 1.48 to add an inventor was granted in this application, however, no power of attorney consistent with the power of attorney granted by the originally named inventive entity has been

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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received. Thus, the addition of the inventor has resulted in the loss of power of attorney in the application. See 37 CFR 1.32(e).

☑ The power of attorney has not been accepted because the party who is giving power of attorney has not been identified. Power of attorney may only be signed by the applicant for patent (37 CFR 1.42) or the patent owner. A patent owner who was not the applicant must appoint any power of attorney in compliance with 37 CFR 3.71 and 3.73. See 37 CFR 1.32(b)(4).

☐ The power of attorney from the inventors has not been accepted because it is a copy from a prior national application for which benefit is claimed and the continuing application names an inventor who was not named as an inventor in the prior application.

☐ The power of attorney from the inventors has not been accepted because the power of attorney must be signed by the applicant for patent. See 37 CFR 1.32(b)(4).

☑ Any request to correct or update the name of the applicant must include an application data sheet (ADS) in compliance with 37 CFR 1.76 specifying the correct or updated name of the applicant in the applicant information section. Any request to change the applicant after an original applicant has been specified under 37 CFR 1.46(b) must include a new ADS in compliance with 37 CFR 1.76 specifying the applicant in the applicant information section and comply with 37 CFR 3.71 and 3.73. See 37 CFR 1.46(c).

Any inquiries regarding this notice should be directed to the Application Assistance Unit at 571-272-4200.

Application Assistance Unit
571-272-4200

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

34055 7590 05/20/2015
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

EXAMINER	
RAO, SAVITHA M	
ART UNIT	PAPER NUMBER
1621	

DATE MAILED: 05/20/2015

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03	7929

TITLE OF INVENTION: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	08/20/2015

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

34055 7590 05/20/2015
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03	7929

TITLE OF INVENTION: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	08/20/2015

EXAMINER	ART UNIT	CLASS-SUBCLASS
RAO, SAVITHA M	1621	424-009200

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee
❑ Publication Fee (No small entity discount permitted)
❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.
❑ Payment by credit card. Form PTO-2038 is attached.
❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

PTOL-85 Part B (10-13) Approved for use through 10/31/2013. OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03	7929

34055 7590 05/20/2015

PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

EXAMINER	
RAO, SAVITHA M	
ART UNIT	PAPER NUMBER
1621	

DATE MAILED: 05/20/2015

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No.	Applicant(s)	
	13/775,000	SCHARSCHMIDT ET AL.	
	Examiner	Art Unit	
	SAVITHA RAO	1621	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *05/11/2015*.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are *1-11*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov .

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☐ Examiner's Amendment/Comment
6. ☒ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/SAVITHA RAO/
Primary Examiner, Art Unit 1621

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION

Claims 1-11 are pending in the instant application.

The terminal disclaimer filed on 05/11/2015 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of US patent 8,404,215 has been reviewed and is accepted. The terminal disclaimer has been recorded.

REASONS FOR ALLOWANCE

In view of the applicants amendments and arguments and terminal disclaimers filed on 5/11/2015, and the following examiners statement of reasons for allowance, claims 1-11 are found to be allowable.

Following a diligent search it was determined that the prior art neither teaches nor provides adequate motivation to arrive at the instantly claimed method A method for adjusting the dosage of glyceryl tri-[4- phenylbutyrate] in a subject being treated for a urea cycle disorder who has previously been administered an initial dosage of glyceryl tri-[4-phenylbutyrate], the method comprising: (a) measuring a fasting plasma ammonia level for the subject; (b) comparing the fasting plasma ammonia level to the upper limit of normal for plasma ammonia level; and (c) administering an adjusted dosage of glyceryl tri-[4-phenylbutyrate], wherein the adjusted dosage is greater than the initial

dosage if the fasting plasma ammonia level is greater than half the upper limit of normal for plasma ammonia level.

Conclusion

Claims 1-11 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAVITHA RAO whose telephone number is (571)270-5315. The examiner can normally be reached on Mon-Fri 7.00 am to 4.00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Melanie McCormick can be reached at 571-272-8037. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/SAVITHA RAO/

Primary Examiner, Art Unit 1621

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	"13775000".rlan. or ("13".src. and "775000".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 07:52
L2	159	((A61K31/216 OR G01N31/221 OR Y10T436/175383).CPC. AND (514/533).CCLS.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 07:53
L3	3	"US 8404215"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 07:54
L4	551	((A61K31/216 OR G01N31/221 OR Y10T436/175383).CPC. AND (514/533 OR 514/432 OR 514/433 OR 514/544 OR 514/570 OR 424/9.2 OR 435/4 OR 436/113).CCLS.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 07:54
L5	233	l4 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 08:10
L6	13	l5 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2015/05/14 08:10
L7	13	"glyceryl tri-[4-phenylbutyrate]"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/05/14 08:10
L8	6	l4 and L7	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/05/14 08:10
S1	0	"13417137".rlan. or ("13".src. and "417137".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 13:46
S2	4	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/15 13:46
S3	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/15 13:46
S4	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/11/15 13:56
S5	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/15 13:56
S6	0	((MASOUD) near2	US-PGPUB;	OR	OFF	2012/11/15

		(MOKHTARANI)).INV.	USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB			13:56
S7	18	("20040229948" | "20060135612" | "4284647" | "6083984" | "20080119554" | "6219567" | "20100008859" | "6050510" | "5968979" | "20100008859" | "6219567").PN.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/11/15 13:57
S8	0	S1 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:08
S9	8	S7 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:08
S10	2	S9 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:08
S11	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:12
S12	4	S11 and PAA	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/15 14:12
S13	4	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/15 14:13
S14	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/11/15 14:13
S15	18	("4284647" | "6083984" | "6050510" | "6219567" | "20040229948" | "20080119554" | "20060135612" | "5968979" | "20100008859").PN.	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/16 07:11
S16	2	S15 and "nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/11/16 07:11
S17	1	("6083984").PN.	USPAT; USOCR	OR	OFF	2012/11/16 07:12
S18	9	((Saul) near2 (Brusilow)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/11/16 07:13
S19	0	"13417137".rlan. or ("13".src. and "417137".ap.)	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S20	4	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 10:56
S21	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 10:56
S22	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/12/20 10:56

S23	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 10:56
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S26	0	S19 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S27	8	S25 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S28	2	S27 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S29	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S30	4	S29 and PAA	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
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S33	18	("4284647" | "6083984" | "6050510" | "6219567" | "20040229948" | "20080119554" | "20060135612" | "5968979" | "20100008859").PN.	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
S34	2	S33 and "nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 10:56
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S36	9	((Saul) near2 (Brusilow)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 10:56
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S38	4	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
S39	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
S40	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	OFF	2012/12/20 16:43

			JPO; DERWENT; IBM_TDB			
S41	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
S42	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/12/20 16:43
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S44	0	S37 and nitrogen	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
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S46	2	S45 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S47	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
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S50	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/12/20 16:43
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S53	1	("6083984").PN.	USPAT; USOCR	OR	OFF	2012/12/20 16:43
S54	9	((Saul) near2 (Brusilow)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
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S57	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43

S58	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/12/20 16:43
S59	0	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
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S64	2	S63 and scavenging	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S65	109	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S66	4	S65 and PAA	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S67	4	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
S68	9	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2012/12/20 16:43
S69	18	("4284647" | "6083984" | "6050510" | "6219567" | "20040229948" | "20080119554" | "20060135612" | "5968979" | "20100008859").PN.	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S70	2	S69 and "nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2012/12/20 16:43
S71	1	("6083984").PN.	USPAT; USOCR	OR	OFF	2012/12/20 16:43
S72	9	((Saul) near2 (Brusilow)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2012/12/20 16:43
S73	49	((BRUCE) near2 (SCHARSCHMIDT)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO;	OR	OFF	2014/12/18 12:08

			DERWENT; IBM_TDB			
S74	127	"nitrogen scavenging"	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2014/12/18 12:08
S75	13	S74 and PAA	US-PGPUB; USPAT; USOCR; DERWENT	OR	OFF	2014/12/18 12:08
S76	11	"glyceryl tri-[4-phenylbutyrate]"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/12/18 12:09
S77	98	((Lee) near2 (Honigberg)).INV.	US-PGPUB; USPAT; USOCR	OR	OFF	2014/12/18 12:11
S78	14	((MASOUD) near2 (MOKHTARANI)).INV.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2014/12/18 12:12
S79	9	("20040229948" | "20060135612" | "20080119554" | "20100008859" | "4284647" | "5968979" | "6060510" | "6083984" | "6219567").PN. OR ("8642012").URPN.	US-PGPUB; USPAT; USOCR	OR	OFF	2014/12/18 13:24

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L9	412	((A61K31/216 OR G01N31/221 OR Y10T436/175383).CPC. AND (514/533 OR 514/432 OR 514/433 OR 514/544 OR 514/570 OR 424/9.2 OR 435/4 OR 436/113).CCLS.)	US-PGPUB; USPAT; UPAD	OR	OFF	2015/05/14 08:21
L10	214	l9 and nitrogen	US-PGPUB; USPAT; UPAD	OR	OFF	2015/05/14 08:21
L11	11	l10 and scavenging	US-PGPUB; USPAT; UPAD	OR	OFF	2015/05/14 08:21
L12	6	l11 and "glyceryl tri-[4-phenylbutyrate]"	US-PGPUB; USPAT; UPAD	OR	OFF	2015/05/14 08:21

5/14/2015 8:35:36 AM
H:\EAST - WKSP\Workspaces\13 applications\13775000.wsp

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13775000	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SAVITHA RAO	1621

CPC							
Symbol						**Type**	**Version**
A61K		31	/	216		F	2013-01-01
Y10T		436	/	175383		A	2015-01-15
G01N		31	/	221		I	2013-01-01
			/				
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			/				
			/				
			/				

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
			/					
			/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	11	
/SAVITHA RAO/ Primary Examiner.Art Unit 1621	05/14/2015	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13775000	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SAVITHA RAO	1621

US ORIGINAL CLASSIFICATION						INTERNATIONAL CLASSIFICATION										
CLASS			SUBCLASS			CLAIMED					NON-CLAIMED					
424			9.2			A	6	1	K	49 / 00 (2006.0)						
CROSS REFERENCE(S)						A	6	1	P	13 / 00 (2006.0)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)															
514	432	433	533	544												
435	4	113														

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 11	
/SAVITHA RAO/ Primary Examiner.Art Unit 1621 (Primary Examiner)	05/14/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13775000	SCHARSCHMIDT ET AL.
	Examiner	**Art Unit**
	SAVITHA RAO	1621

☒ Claims renumbered in the same order as presented by applicant	☐ CPA	☒ T.D.	☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 11	
/SAVITHA RAO/ Primary Examiner.Art Unit 1621 (Primary Examiner)	05/14/2015 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	13775000	SCHARSCHMIDT ET AL.
	Examiner	Art Unit
	SAVITHA RAO	1621

CPC- SEARCHED		
Symbol	Date	Examiner
A61K31/216 OR G01N31/221 OR Y10T436/175383	5/14/2015	SR

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner
424	9.2	5/14/2015	SR
514	432, 433, 544, 570, 533	5/14/2015	SR
436	4,113	5/14/2015	SR

SEARCH NOTES		
Search Notes	Date	Examiner
eaST search (See attached)	12/21/2014	SR
Inventor search in EAST and PALM	12/21/2014	SR
Reviewed STN searches from the Parent application, further NPL search in Google	12/21/2014	SR
updated EAST search (See attached)	5/14/2015	SR
updated inventor search in EAST	5/14/2015	SR
updated NPL and STN search	5/14/2015	SR

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner
A61K	31/216	5/14/2015	SR
G01N	31/221	5/14/2015	SR
Y10T	436/175383	5/14/2015	SR
424	9.2	5/14/2015	SR

	/SAVITHA RAO/ Primary Examiner.Art Unit 1621

INTERFERENCE SEARCH			
US Class/ CPC Symbol	**US Subclass / CPC Group**	**Date**	**Examiner**
514	533, 432, 433, 544, 570	5/14/2015	SR
435	4, 113	5/14/2015	SR

	/SAVITHA RAO/ Primary Examiner.Art Unit 1621

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/775,000	02/22/2013	Bruce Scharschmidt	079532-8003.US03

CONFIRMATION NO. 7929

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

MISCELLANEOUS NOTICE

OC000000075263036

Date Mailed: 05/20/2015

A communication which cannot be delivered in electronic form has been mailed to the applicant.

Doc Code: PA..
Document Description: Power of Attorney

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Application Number	13/775,000
Filing Date	February 22, 2013
First Named Inventor	Bruce Scharschmidt
Title	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
Art Unit	1621
Examiner Name	Rao, Savitha M.
Attorney Docket Number	HOR0026-201D1-US

SIGNATURE of Applicant or Patent Practitioner

Signature	/Dennis A. Bennett/	Date (Optional)	
Name	Dennis A. Bennett	Registration Number	34547
Title (if Applicant is a juristic entity)	Attorney		
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.

☑ *Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc Code: PA..
Document Description: Power of Attorney

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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

☑ I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 101325

OR

☐ I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:

☑ The address associated with the above-mentioned Customer Number

OR

☐ The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

Horizon Therapeutics, Inc.

☐ Inventor or Joint Inventor (title not required below)

☐ Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

☑ Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

☐ Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature		Date (Optional)	5/1/15
Name	Brian R. Beeler		
Title	SVP, Legal		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

☐ Total of forms are submitted.

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STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: Horizon Therapeutics, Inc.

Application No./Patent No.: 13/775,000 Filed/Issue Date: February 22, 2013

Titled: METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Horizon Therapeutics, Inc., a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

☐ The extent (by percentage) of its ownership interest is _____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

B. ☑ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Bruce Scharschmidt et al. To: Hyperion Therapeutics, Inc.

The document was recorded in the United States Patent and Trademark Office at
Reel 035361, Frame 0777, or for which a copy thereof is attached.

2. From: Hyperion Therapeutics, Inc. To: Horizon Therapeutics, Inc.

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STATEMENT UNDER 37 CFR 3.73(c)

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6. From: _____ To: _____

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☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Dennis A. Bennett/	May 29, 2015
Signature	Date
Dennis A. Bennett	34547
Printed or Typed Name	Title or Registration Number

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, arecord may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from thissystem of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	22481929
Application Number:	13775000
International Application Number:	
Confirmation Number:	7929
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Dennis A. Bennett/Vicki Truman
Filer Authorized By:	Dennis A. Bennett
Attorney Docket Number:	079532-8003.US03
Receipt Date:	29-MAY-2015
Filing Date:	22-FEB-2013
Time Stamp:	12:50:02
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	20150529_SuppADS.pdf	123521 72f63bf4b2fc14a37fbbd99698deb120a041e96a	no	7
Warnings:					
Information:					

This is not an USPTO supplied ADS fillable form					
2	Power of Attorney	20150529_POA1.pdf	259835 d21e83e0bae69f6ca9bd0b0b48e491a986ea05de	no	2
Warnings:					
Information:					
3	Assignee showing of ownership per 37 CFR 3.73	20150529_373_Statement.pdf	119656 b4b6a12fbe73cc7e0b30a007ed92dac894a3e9ec	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	503012		

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	~~79532.8003.US03~~ HOR0026-201D1-US
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
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Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Bruce		Scharschmidt	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	45 St. Francis Boulevard		
Address 2			
City	San Francisco	State/Province	CA
Postal Code	94127	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Masoud		Mokhtarani	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Walnut Creek	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	725 Castle Rock Road		
Address 2			
City	Walnut Creek	State/Province	CA
Postal Code	94598	Country	US

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Correspondence Information:

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PTO/AIA/14 (07-14)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	~~34055~~ 101325		
Email Address	~~patentprocurement@perkinscoie.com~~ admin@globalpatentgroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS		
Attorney Docket Number	~~79532.8003.US03~~ HOR0026-201D1-US	**Small Entity Status Claimed**	☒
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	3	**Suggested Figure for Publication (if any)**	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
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Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	34055		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Division of	13417137	2012-03-09
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13417137	Claims benefit of provisional	61542100	2011-09-30
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13417137	Claims benefit of provisional	61564668	2011-11-29

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This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☒ Authorization to Permit Access to the Instant Application by the Participating Offices

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In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	79532.8003.US03
	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee ○ Legal Representative under 35 U.S.C. 117 ○ Joint Inventor

○ Person to whom the inventor is obligated to assign. ○ Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	~~HYPERION THERAPEUTICS, INC.~~ Horizon Therapeutics, Inc.

Mailing Address Information For Applicant:

Address 1	~~601 Gateway Blvd.~~ 533 Bryant		
Address 2	~~Suite 200~~ Suite #6		
City	~~South San Francisco~~ Palo Alto	**State/Province**	CA
Country	US	Postal Code	~~94080~~ 94301
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

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Assignee 1

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If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

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	Application Number	
Title of Invention	METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country [i]		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/Dennis A. Bennett/			Date (YYYY-MM-DD)	2015-05-29
First Name	Dennis	Last Name	Bennett	Registration Number	34547

Additional Signature may be generated within this form by selecting the Add button.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/775,000	02/22/2013	1621	828	HOR0026-201D1-US	11	3

CONFIRMATION NO. 7929

REPLACEMENT FILING RECEIPT

101325
GLOBAL PATENT GROUP - HOR
1005 NORTH WARSON ROAD
SUITE 404
SAINT LOUIS, MO 63132

Date Mailed: 06/03/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

Applicant(s)

Horizon Therapeutics, Inc., Palo Alto, CA;

Power of Attorney: The patent practitioners associated with Customer Number 101325

Domestic Priority data as claimed by applicant

This application is a DIV of 13/417,137 03/09/2012 PAT 8404215 *
which claims benefit of 61/542,100 09/30/2011
and claims benefit of 61/564,668 11/29/2011
(*)Data provided by applicant is not consistent with PTO records.

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Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/775,000**
Projected Publication Date: Not Applicable

Non-Publication Request: No

Early Publication Request: No
** **SMALL ENTITY** **
Title

METHODS OF THERAPEUTIC MONITORING OF NITROGEN SCAVENGING DRUGS

Preliminary Class

424

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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(51) International Patent Classification 5 : A61K 49/02 — A1

(11) International Publication Number: WO 94/22494

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(21) International Application Number: PCT/US94/03256

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(30) Priority Data:

08/040,336	30 March 1993 (30.03.93)	US
08/218,861	28 March 1994 (28.03.94)	US

(71) Applicant: THE DU PONT MERCK PHARMACEUTICAL COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors: DeGRADO, William, Frank; 502 Bancroft Road, Moylan, PA 19063-4207 (US). MOUSA, Shaker, Ahmed; 4 Linden Circle, Lincoln University, PA 19352-8933 (US). SWORIN, Michael; 19 Mary Ella Drive, Newark, DE 19711-5679 (US). BARRETT, John, Andrew; 46 Fox Run, West Groton, MA 01450 (US). EDWARDS, David, Scott; 123 Farms Drive, Burlington, MA 01803 (US). HARRIS, Thomas, David; 56 Zion Hill Road, Salem, NH 03079 (US). RAJOPADHYE, Milind; 21 Honeysuckle Road, Westford, MA 01886-4038 (US). LIU, Shuang; 17 Judith Road, Chelmsford, MA 01824-4742 (US).

(74) Agents: BOUDREAUX, Gerald, J. et al.; The du Pont Merck Pharmaceutical Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

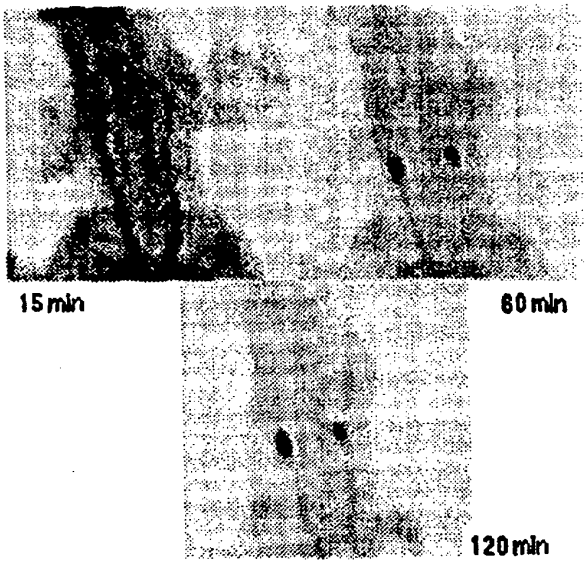
(81) Designated States: AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: RADIOLABELED PLATELET GPIIb/IIIa RECEPTOR ANTAGONISTS AS IMAGING AGENTS FOR THE DIAGNOSIS OF THROMBOEMBOLIC DISORDERS

(57) Abstract

This invention provides novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems which act as antagonists of the platelet glycoprotein IIb/IIIa complex; to methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi; to novel reagents for the preparation of said radiopharmaceuticals; and to kits comprising said reagents.



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GA	Gabon				

TITLE

Radiolabeled Platelet GPIIb/IIIa Receptor Antagonists As Imaging Agents For The Diagnosis Of Thromboembolic Disorders

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of our copending application U.S.S.N. 08/040,336 filed March 30, 1993, the disclosure of which is hereby incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems; to methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi; to novel reagents for the preparation of said radiopharmaceuticals; and to kits comprising said reagents.

BACKGROUND OF THE INVENTION

The clinical recognition of venous and arterial thromboembolic disorders is unreliable, lacking in both sensitivity and specificity. In light of the potentially life threatening situation, the need to rapidly diagnose thromboembolic disorders using a non invasive method is an unmet clinical need. Platelet activation and resulting aggregation has been shown to be associated with various pathophysiological conditions including cardiovascular and cerebrovascular thromboembolic disorders such as unstable angina, myocardial infarction, transient ischemic attack, stroke, atherosclerosis and diabetes. The contribution

of platelets to these disease processes stems from their ability to form aggregates, or platelet thrombi, especially in the arterial wall following injury. See generally, Fuster et al., JACC, Vol. 5, No. 6, pp. 175B-183B (1985); Rubenstein et al., Am. Heart J., Vol. 102, pp. 363-367 (1981); Hamm et al., J. Am. Coll. Cardiol., Vol. 10, pp. 998-1006 (1987); and Davies et al., Circulation, Vol. 73, pp. 418-427 (1986). Recently, the platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa), has been identified as the membrane protein which mediates platelet aggregation by providing a common pathway for the known platelet agonists. See Philips et al., Cell, Vol. 65, pp. 359-362 (1991).

Platelet activation and aggregation is also thought to play a significant role in venous thromboembolic disorders such as venous thrombophlebitis and subsequent pulmonary emboli. It is also known that patients whose blood flows over artificial surfaces, such as prosthetic synthetic cardiac valves, are at risk for the development of platelet plugs, thrombi and emboli. See generally Fuster et al., JACC, Vol. 5, No. 6, pp. 175B-183B (1985); Rubenstein et al., Am. Heart J., Vol. 102, pp. 363-367 (1981); Hamm et al., J. Am. Coll. Cardiol., Vol. 10, pp. 998-1006 (1987); and Davies et al., Circulation, Vol. 73, pp. 418-427 (1986).

A suitable means for the non-invasive diagnosis and monitoring of patients with such potential thromboembolic disorders would be highly useful, and several attempts have been made to develop radiolabeled agents targeted to platelets for non-invasive radionuclide imaging. For example, experimental studies have been carried out with 99mTc monoclonal antifibrin antibody for diagnostic imaging of arterial thrombus. See Cerqueira et al., Circulation, Vol., 85, pp. 298-304

(1992). The authors report the potential utility of such agents in the imaging of freshly formed arterial thrombus. Monoclonal antibodies labeled with 131I and specific for activated human platelets have also been reported to have potential application in the diagnosis of arterial and venous thrombi. However, a reasonable ratio of thrombus to blood (target/background) was only attainable at 4 hours after the administration of the radiolabeled antibody. See Wu et al., Clin. Med. J., Vol. 105, pp. 533-559 (1992). The use of 125I, 131I, 99mTc, and 111In radiolabeled 7E3 monoclonal antiplatelet antibody in imaging thrombi has also been recently discussed. Coller et al., PCT Application Publication No. WO 89/11538 (1989). The radiolabeled 7E3 antibody has the disadvantage, however, of being a very large molecular weight molecule. Other researchers have employed enzymatically inactivated t-PA radioiodinated with 123I, 125I and 131I for the detection and the localization of thrombi. See Ordm et al., Circulation, Vol. 85, pp. 288-297 (1992). Still other approaches in the radiologic detection of thromoboembolisms are described, for example, in Koblik et al., Semin. Nucl. Med., Vol. 19, pp. 221-237 (1989).

Arterial and venous thrombus detection and localization is of critical importance in accurately diagnosing thromboembolic disorders and determining proper therapy. New and better radiolabeled agents for non-invasive radionuclide imaging to detect thrombi are needed. The present invention is directed to this important end.

SUMMARY OF THE INVENTION

This invention provides novel radiopharmaceuticals that are radiolabeled cyclic compounds containing carbocyclic or heterocyclic ring systems which act as

antagonists of the platelet glycoprotein IIb/IIIa complex. It also provides methods of using said radiopharmaceuticals as imaging agents for the diagnosis of arterial and venous thrombi. It further provides novel reagents for the preparation of said radiopharmaceuticals. It further provides kits comprising said reagents.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a. Illustrated are typical images of the radiopharmaceutical compound of Example 12 administered at 1 mCi/Kg,i.v. in a canine deep venous thrombosis model. In this model thrombi were formed in the jugular veins during a period of stasis which was followed by reflow. The compounds were administered beginning at reflow. Depicted is the uptake in a rapidly growing venous thrombus at 15, 60 and 120 min post-administration.

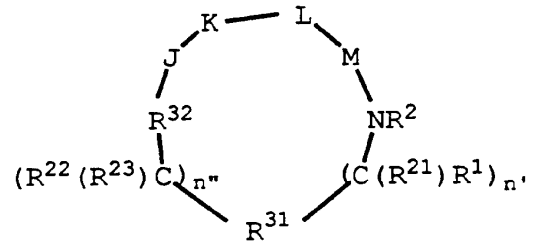
Figure 1b. Illustrated are typical images of the radiopharmaceutical compound of Example 19 administered at 1 mCi/Kg,i.v. in a canine deep venous thrombosis model. In this model thrombi were formed in the jugular veins during a period of stasis which was followed by reflow. The compounds were administered beginning at reflow. Depicted is the uptake in a rapidly growing venous thrombus at 15, 60 and 120 min post-administration.

DETAILED DESCRIPTION OF THE INVENTION

[1] The present invention is directed to novel reagents for preparing a radiopharmaceutical of formulae:

$(QL_n)_dC_h$; $(Q)_{d'}L_n-C_h$,

wherein, d is 1-3, d' is 2-20, L_n is a linking group, C_h is a metal chelator, and Q is a compound of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof, wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n; a heterocyclic ring system, optionally substituted with 0-4 R^{10} or R^{10a}, and optionally bearing a bond to L_n;

R^{32} is selected from:

-C(=O)-;
-C(=S)-
$-S(=O)_2-$;
-S(=O)-;
$-P(=Z)(ZR^{13})-$;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:

hydrogen;

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

$-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, $(C_1-C_5$ alkyl$)NHR^{16}$;

$-(C_0-C_6$ alkyl$)X$;

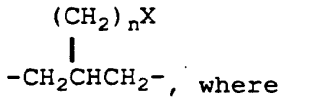
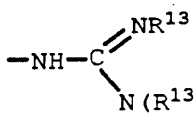

, where q is independently 0,1;


;

$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form


, where n = 0,1 and X is  ;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

R^{16} is selected from:

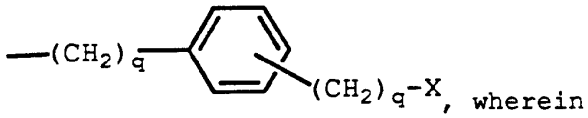
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is a D-isomer or L-isomer amino acid of structure
$-N(R^6)CH(R^7)C(=O)-$, wherein:

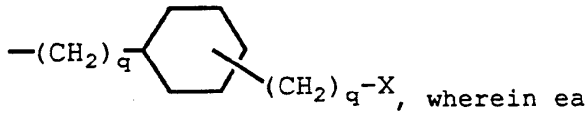
R^6 is H or C_1-C_8 alkyl;

R^7 is selected from:

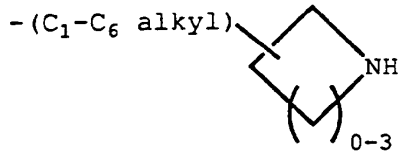
$-(C_1$-C_7 alkyl)X;



wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;



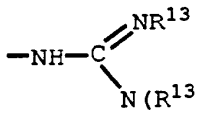
wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



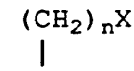
$-(CH_2)_mO-(C_1-C_4$ alkyl)-X, where m = 1 or 2;

$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl)-X, where m = 1 or 2 and p' = 0-2; and

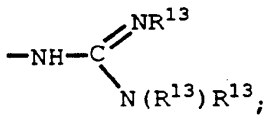
X is selected from:


; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form


, wherein each q is independently 1 or 2 and wherein

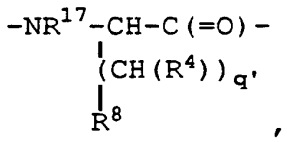
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, N(C_1-C_3 alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or

C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:

a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;

a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;

a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

[2] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n"}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

[3] Included in the present invention are those reagents in [1] above, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;
n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;

n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

[4] Included in the present invention are those reagents in [1] above, wherein:
wherein R^6 is methyl, ethyl, or propyl.

[5] Included in the present invention are those reagents in [1] above, wherein:

R^{32} is selected from:
-C(=O)-;
-C(=S)-
$-S(=O)_2-$;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11},
C_2-C_8 alkenyl substituted with 0-2 R^{11},
C_2-C_8 alkynyl substituted with 0-2 R^{11},
C_3-C_8 cycloalkyl substituted with 0-2 R^{11},
C_6-C_{10} bicycloalkyl substituted with 0-2 R^{11};

a bond to L_n;

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, NO_2;

R^1 and R^{21} can alternatively join to form a 5-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form

a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-CH_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2;

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_1-C_4 alkyl (substituted with $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13}$, or $-S(=O)R^{13a}$)

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

R^3 is H or CH_3;

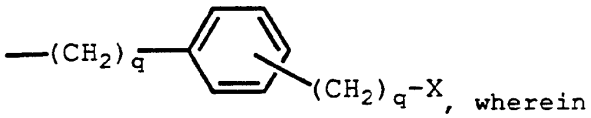
R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sNHR^{16}$, where s = 3-5;

a bond to L_n;

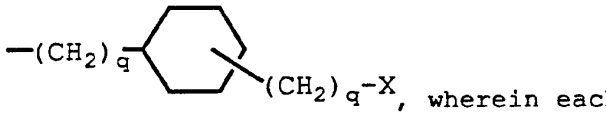
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

R^7 is selected from:

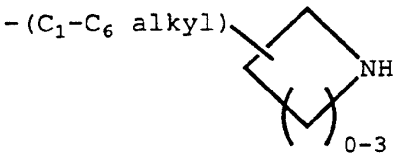
$-(C_1-C_7$ alkyl$)X$;



wherein each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;



wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



$-(CH_2)_mO-(C_1-C_4$ alkyl)-X, where m = 1 or 2;

$-(CH_2)_mS-(C_1-C_4$ alkyl)-X, where m = 1 or 2; and

X is selected from:
$-NH-C(=NH)(NH_2)$, $-NHR^{13}$, $-C(=NH)(NH_2)$, $-SC(NH)-NH_2$;

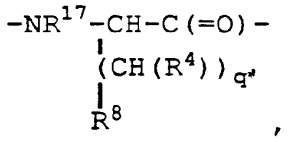
R^6 and R^7 can alternatively be taken together to form

$-CH_2CH(CH_2)_nX-CH_2-$, where
n = 0 or 1 and X is $-NH_2$ or $-NH-C(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, $N(C_1-C_3$ alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-NR^{13}R^{14}$, or
C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms

and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O.

[6] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln;

(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-3 R^{10} or R^{10a}, and optionally bearing a bond to Ln; or

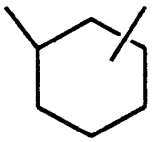
(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-3 R^{10}

or R^{10a}, and optionally bearing a bond to Ln.

[7] Included in the present invention are those reagents in [1] above, wherein:

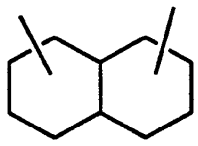
R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formulae:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond, and wherein said carbocyclic ring is substituted with 0-3 R^{10}, and optionally bears a bond to Ln;

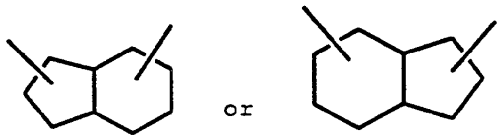
(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic

ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n;

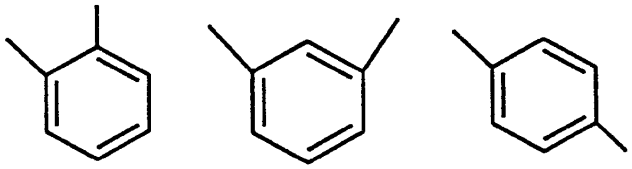
(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



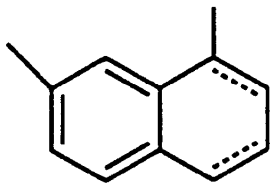
wherein any of the bonds forming the carbocyclic ring may be a single or double bond, wherein said carbocyclic ring is substituted independently with 0-4 R^{10}, and optionally bears a bond to L_n.

[8] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



; or



wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and optionally bears a bond to L_n;

n" is 0 or 1; and

n' is 0-2.

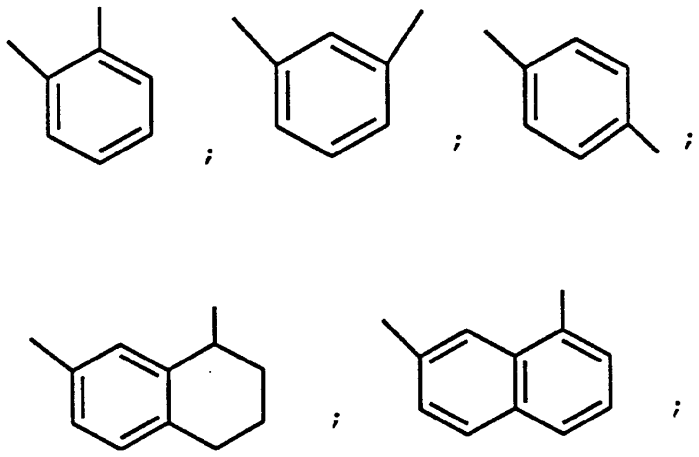
[9] Included in the present invention are those reagents in [1] above, wherein:

R^1 and R^{22} are independently selected from: phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4

alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

[10] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is selected from:



wherein R^{31} may be independently substituted with 0-3 R^{10} or R^{10a}, and may optionally bear a bond to L_n;

R^{32} is $-C(=O)-$;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy; and a bond to L_n;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; and a bond to L_n; or

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

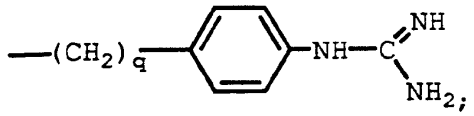
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is



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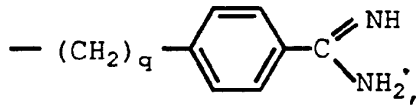
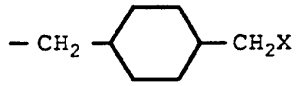

, where q = 0 or 1;

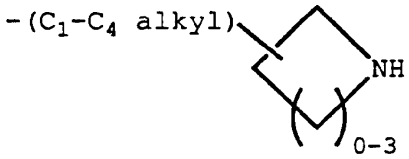
$-(CH_2)_rX$, where r = 3-6;


;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)-NH-$(C_1-C_6$ alkyl);

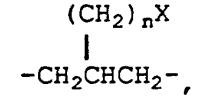


$-(CH_2)_m-O-(C_1-C_4$ alkyl)-NH-$(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)-NH-$(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form

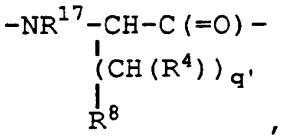

, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



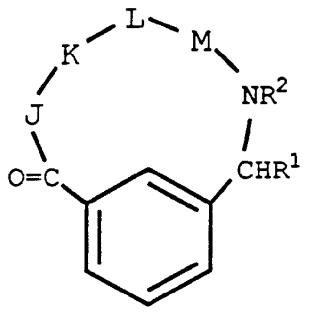
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[11] Included in the present invention are those reagents in [1] above, wherein Q is a 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be substituted with 0-3 R^{10}, and may optionally bear a bond to L_n;

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_1-C_4)alkyl, or a bond to L_n;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5, or a bond to L_n;

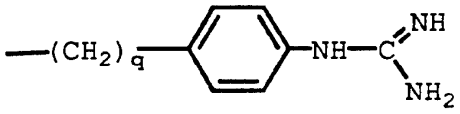
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

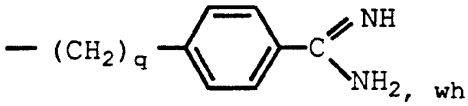
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

R^7 is:



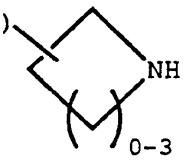


where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3$-C_7 alkyl$)$-NH-$(C_1$-C_6 alkyl$)$

$-(C_1$-C_4 alkyl$)$ 

$-(CH_2)_m$-O-$(C_1$-C_4 alkyl$)$-NH-$(C_1$-C_6 alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl$)$, where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

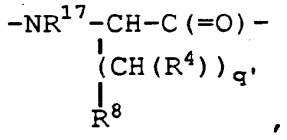
R^6 and R7 are alternatively be taken together to form

where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

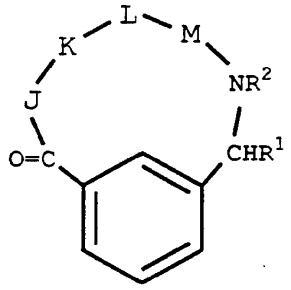
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said

heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[12] Included in the present invention are those reagents in [1] above, wherein Q is 1,3-disubstituted phenyl compound of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5; or a bond to L_n;

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

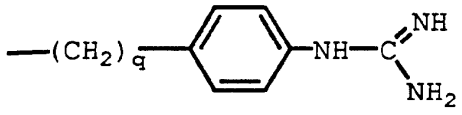
R^{16} is selected from:

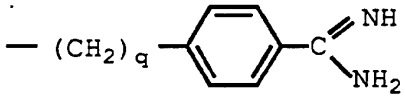
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_3-C_8 alkyl;

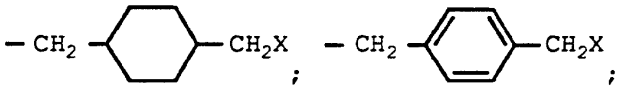
R^7 is





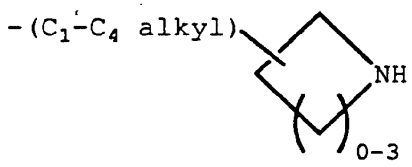
where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl$)$-NH-$(C_1$-C_6 alkyl$)$



$-(CH_2)_m-O-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

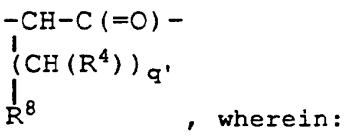
$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2H$ or $-SO_3R^{13}$.

[13] Included in the present invention are those reagents in [1] above, wherein:

the phenyl ring in formula (II) bears a bond to L_n, and may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form -$(CH_2)_{2-5}$- or -$(CH_2)O(CH_2)$-;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula -$N(R^3)CH(R^5)C(=O)$-, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

R^{16} is selected from:

an amine protecting group;

1-2 amino acids;

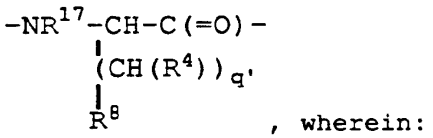
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is

$-CO_2H$;

$-SO_3H$.

[14] Included in the present invention are those reagents in [1] above, wherein:

the phenyl ring in formula (II) bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, β-Ala, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[15] Included in the present invention are those reagents in [1] above, wherein:

R^{31} is a phenyl ring and bears a bond to L_n;

R^1 and R^2 are independently selected from H, methyl;

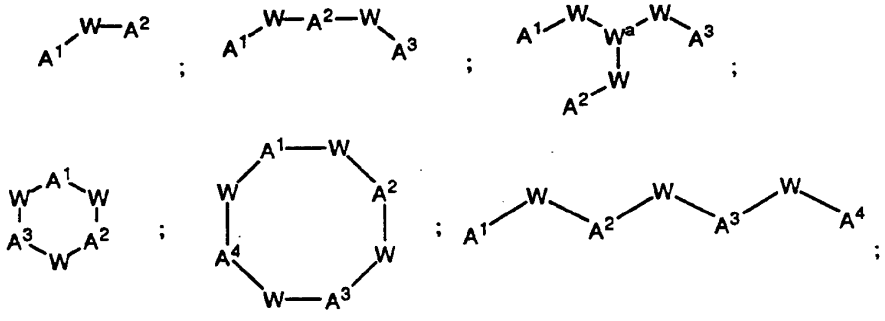
J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

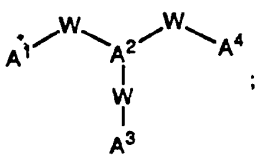
K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[16] Included in the present invention are those reagents in [1]-[15] above, wherein C_h is selected from the group:

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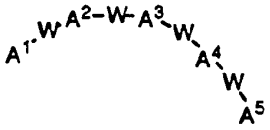
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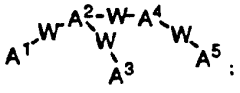
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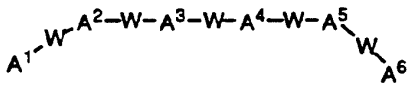
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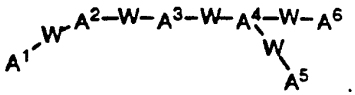
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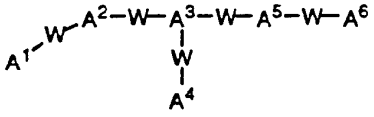
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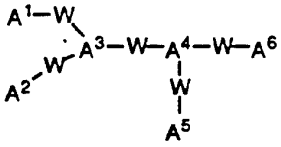
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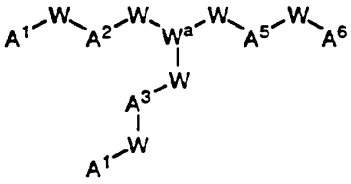
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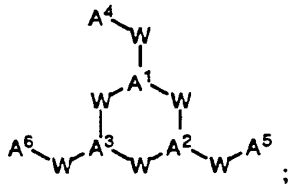
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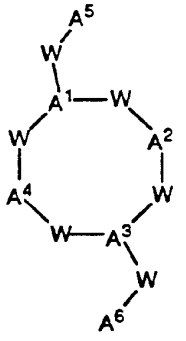
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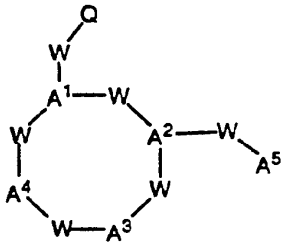
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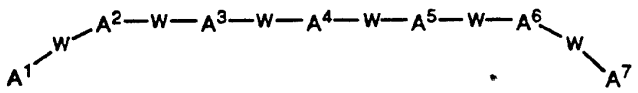

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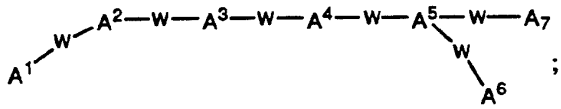

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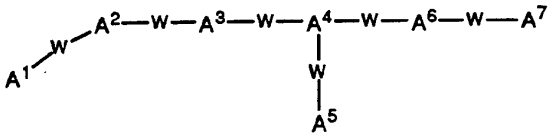

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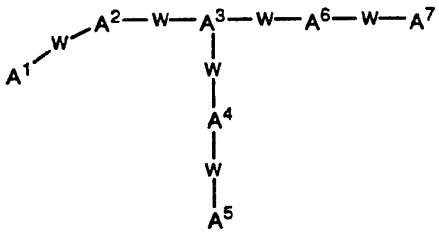

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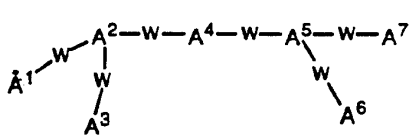
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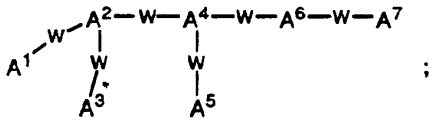
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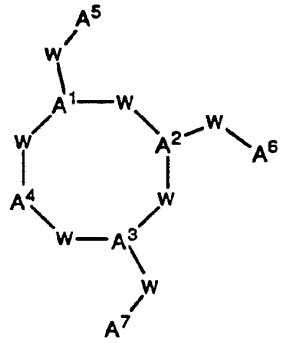
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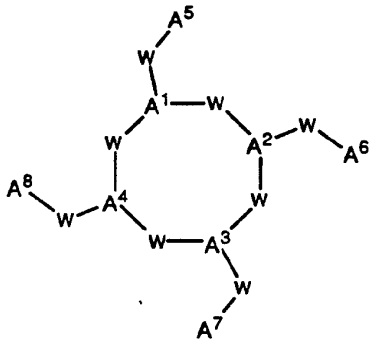
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wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), O, OH, $PR^{42}R^{43}$, $P(O)R^{42}R^{43}$, $P(S)R^{42}R^{43}$, $P(NR^{44})R^{42}R^{43}$;

W is a bond, CH, or a spacer group selected from the group: C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52} and alkaryl substituted with 0-3 R^{52};

W^a is a C_1-C_{10} alkyl group or a C_3-C_{14} carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, aryl substituted with 0-3 R^{52}, cycloaklyl substituted with 0-3 R^{52}, heterocycloalkyl substituted with 0-3 R^{52}, aralkyl substituted with 0-3 R^{52}, alkaryl substituted with 0-3 R^{52}substituted with 0-3 R^{52} and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form $=C(C_1\text{-}C_3$ alkyl)$(C_1\text{-}C_3$ alkyl);

R^{52} is independently selected at each occurrence from the group: a bond to L_n, =O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, -CHO, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$, $-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$,

$-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$,
2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl,

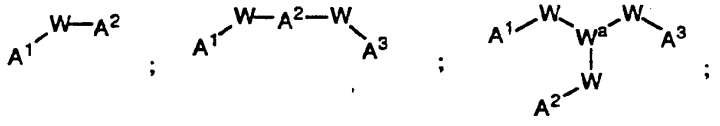
aryl substituted with 0-2 R^{53},

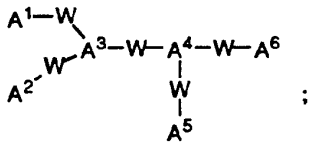
a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O;

R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl, phenyl, benzyl, C_1-C_6 alkoxy, halide, nitro, cyano, and trifluoromethyl; and

Pg is a thiol protecting group capable of being displaced upon reaction with a radionuclide.

[17] Included in the present invention are those reagents in [1]-[15] above, wherein C_h is selected from the group:

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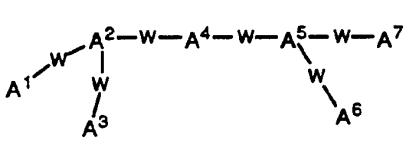
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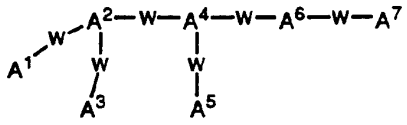
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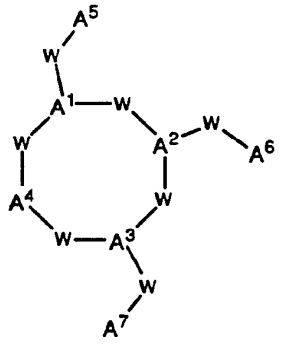
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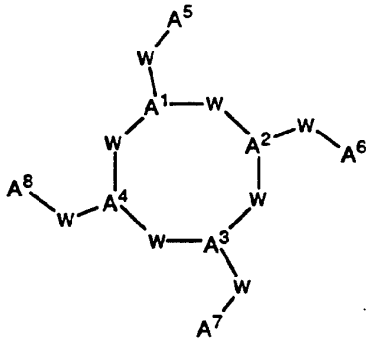
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wherein:

A^1, A^2, A^3, A^4, A^5, A^6, and A^7 are independently selected at each occurrence from the group: $NR^{40}R^{41}$, S, SH, S(Pg), OH;

W is a bond, CH, or a spacer group selected from the group: C_1-C_3 alkyl substituted with 0-3 R^{52};

W^a is a methylene group or a C_3-C_6 carbocycle;

R^{40}, R^{41}, R^{42}, R^{43}, and R^{44} are each independently selected from the group: a bond to L_n, hydrogen, C_1-C_{10} alkyl substituted with 0-3 R^{52}, and an electron, provided that when one of R^{40} or R^{41} is an electron, then the other is also an electron, and provided that when one of R^{42} or R^{43} is an electron, then the other is also an electron;

additionally, R^{40} and R^{41} may combine to form, $=C(C_1\text{-}C_3$ alkyl$)(C_1\text{-}C_3$ alkyl$)$;

R^{52} is independently selected at each occurrence from the group: a bond to L_n, $=O$, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{53}$, $-C(=O)R^{53}$, $-C(=O)N(R^{53})_2$, $-CHO$, $-CH_2OR^{53}$, $-OC(=O)R^{53}$, $-OC(=O)OR^{53a}$, $-OR^{53}$, $-OC(=O)N(R^{53})_2$, $-NR^{53}C(=O)R^{53}$, $-NR^{54}C(=O)OR^{53a}$, $-NR^{53}C(=O)N(R^{53})_2$, $-NR^{54}SO_2N(R^{53})_2$, $-NR^{54}SO_2R^{53a}$, $-SO_3H$, $-SO_2R^{53a}$, $-SR^{53}$, $-S(=O)R^{53a}$, $-SO_2N(R^{53})_2$, $-N(R^{53})_2$, $-NHC(=NH)NHR^{53}$, $-C(=NH)NHR^{53}$, $=NOR^{53}$, NO_2, $-C(=O)NHOR^{53}$, $-C(=O)NHNR^{53}R^{53a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

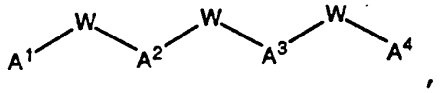
R^{53}, R^{53a}, and R^{54} are independently selected at each occurrence from the group: a bond to L_n, C_1-C_6 alkyl.

[18] Included in the present invention are those reagents in [1]-[15] above, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

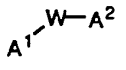
C_h is selected from:



wherein:

A^1 and A^4 are SH or SPg;
A^2 and A^3 are NR^{41};
W is independently selected from the group:
CHR^{52}, CH_2CHR^{52}, $CH_2CH_2CHR^{52}$ and $CHR^{52}C{=}O$; and
R^{41} and R^{52} are independently selected from hydrogen and a bond to L_n,

and,



wherein:

A^1 is NH_2 or $N{=}C(C_1\text{-}C_3$ alkyl$)(C_1\text{-}C_3$ alkyl);
W is a bond;

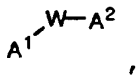
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the heterocycle is selected from the group: pyridine, pyrazine, proline, furan, thiofuran, thiazole, and diazine, and R^{52} is a bond to L_n.

[19] Included in the present invention are those reagents in [1]-[15] above, of formula:

$$(QL_n)_dC_h,$$

wherein d is 1; and

wherein C_h is:



wherein:

A^1 is NH_2 or $N=C(C_1\text{-}C_3$ alkyl$)(C_1\text{-}C_3$ alkyl$)$;
W is a bond;
A^2 is NHR^{40}, wherein R^{40} is heterocycle substituted with R^{52}, wherein the heterocycle is selected from pyridine and thiazole, and R^{52} is a bond to L_n.

[20] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

a bond between Q and C_h; or,
a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h''}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g''}-$;
M^2 is $-(CR^{55}R^{56})_{g''}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;
g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};

(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, $C\equiv N$, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R^{57} is independently selected at each occurrence from the group: O, NR^{58}, C=O, $C(=O)O$, $OC(=O)O$, $C(=O)N$-, $C=NR^{58}$, S, SO, SO_2, SO_3, $NHC(=O)$, $(NH)_2C(=O)$, $(NH)_2C=S$; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[21] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

a compound of formula:

$$M^1-[Y^1(CR^{55}R^{56})_h(Z^1)_{h''}Y^2]_{h'}-M^2$$

wherein:

M^1 is $-[(CH_2)_gZ^1]_{g'}-(CR^{55}R^{56})_{g''}-$;
M^2 is $-(CR^{55}R^{56})_{g''}-[Z^1(CH_2)_g]_{g'}-$;
g is independently 0-10;

g' is independently 0-1;
g" is 0-10;
h is 0-10;
h' is 0-10;
h" is 0-1
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

Z^1 is independently selected at each occurrence from a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system, substituted with 0-4 R^{57}; a heterocyclic ring system, optionally substituted with 0-4 R^{57};

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-,

C≡N, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, C(=O)O, OC(=O)O, C(=O)N-, $C=NR^{58}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[22] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-$,

wherein:

g" is 1-10;
h is 0-10;
h' is 1-10;
Y^1 and Y^2, at each occurrence, are independently selected from:

a bond, O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl substituted with 0-5 R^{57};
(C_1-C_{10} alkyl)aryl wherein the aryl is substituted with 0-5 R^{57};

R^{57} is independently selected at each occurrence from the group: hydrogen, OH, NHR^{58}, $C(=O)R^{58}$, $OC(=O)R^{58}$, $OC(=O)OR^{58}$, $C(=O)OR^{58}$, $C(=O)NR^{58}$-, $C\equiv N$, SR^{58}, SOR^{58}, SO_2R^{58}, $NHC(=O)R^{58}$, $NHC(=O)NHR^{58}$, $NHC(=S)NHR^{58}$; or, alternatively, when attached to an additional molecule Q, R57 is independently selected at each occurrence from the group: O, NR^{58}, C=O, $C(=O)O$, $OC(=O)O$, $C(=O)N$-, $C=NR^{58}$, S, SO, SO_2, SO_3, $NHC(=O)$, $(NH)_2C(=O)$, $(NH)_2C=S$, and R^{57} is attached to an additional molecule Q; and,

R^{58} is independently selected at each occurrence from the group:hydrogen; C_1-C_6 alkyl; benzyl, and phenyl.

[23] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$-(CR^{55}R^{56})_{g''}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g''}-$,

wherein:

g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

O, NR^{56}, C=O, C(=O)O, OC(=O)O, C(=O)NH-, $C=NR^{56}$, S, SO, SO_2, SO_3, NHC(=O), $(NH)_2C(=O)$, $(NH)_2C=S$;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen;
C_1-C_{10} alkyl;
(C_1-C_{10} alkyl)aryl.

[24] Included in the present invention are those reagents in [1]-[15] above, wherein Ln is:

$-(CR^{55}R^{56})_{g"}-[Y^1(CR^{55}R^{56})_hY^2]_{h'}-(CR^{55}R^{56})_{g"}-$,

wherein:

g" is 1-5;
h is 0-5;
h' is 1-5;
Y^1 and Y^2, at each occurrence, are independently selected from:

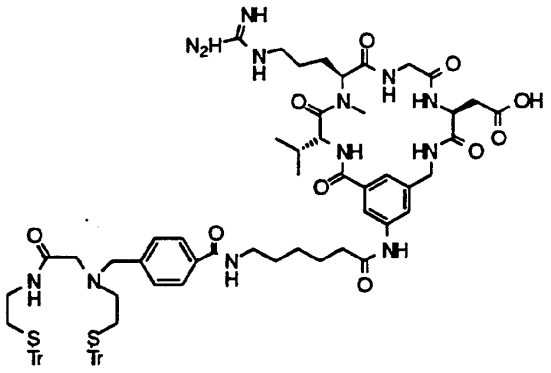
O, NR^{56}, C=O, C(=O)O, OC(=O)O,

C(=O)NH-, C=NR^{56}, S,
NHC(=O), (NH)$_2$C(=O), (NH)$_2$C=S;

R^{55} and R^{56} are independently selected at each occurrence from:

hydrogen.

[25] Included in the present invention are those reagents in [1] above, which are:



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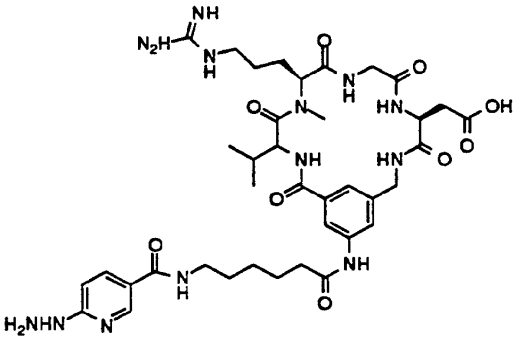

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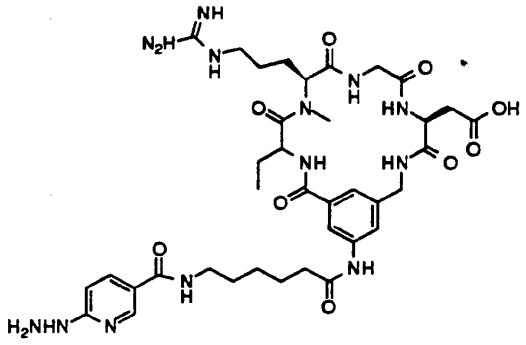

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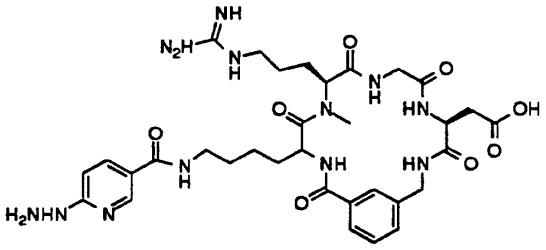

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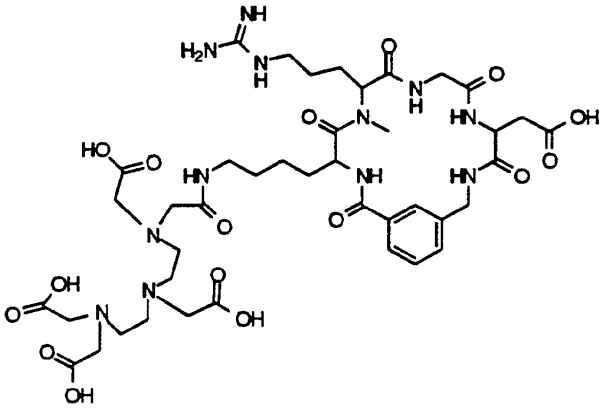

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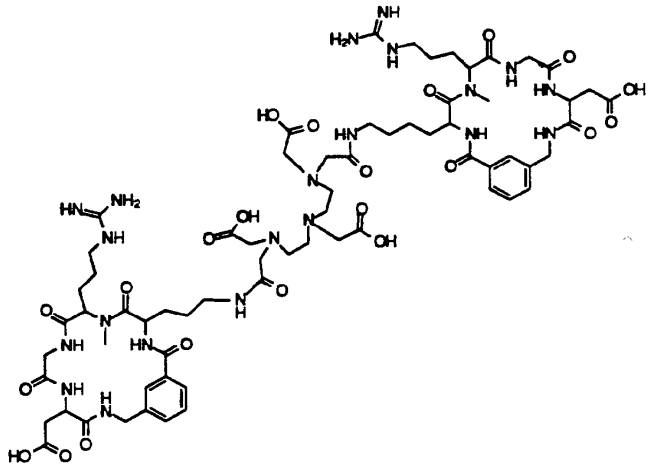


[26] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [23].

[27] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [24].

[28] Also included in the present invention is a kit for preparing a radiopharmaceutical comprising a predetermined quantity of a sterile, pharmaceutically acceptable reagent of [25].

[29] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [1]-[15] and a radionuclide selected

from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[30] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [16] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[31] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [17] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[32] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [18] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[33] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [19] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

[34] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [20] and a radionuclide selected from the group ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl.

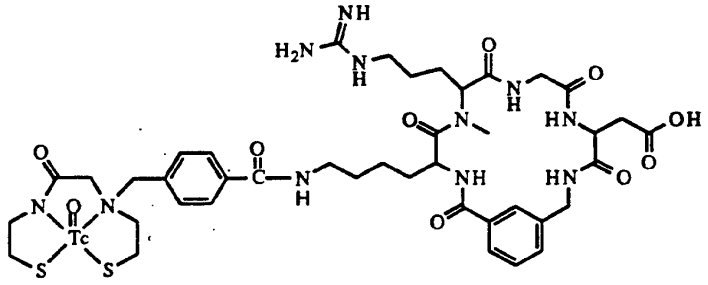
[35] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [21] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[36] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [22] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[37] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [23] and a radionuclide selected from the group ^{99m}Tc, ^{111}In, and ^{62}Cu.

[38] Also included in the present invention is a radiopharmaceutical comprising a complex of a reagent of [24] and a radionuclide selected from the group ^{99m}Tc, and ^{111}In.

[39] Also included in the present invention are the radiopharmaceuticals of [29] which are:



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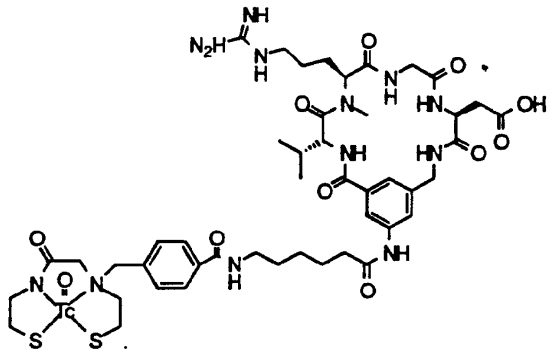

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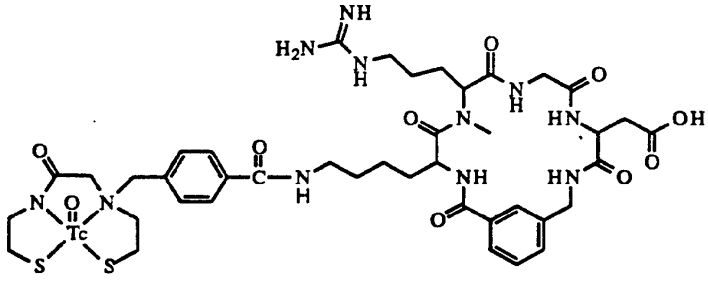

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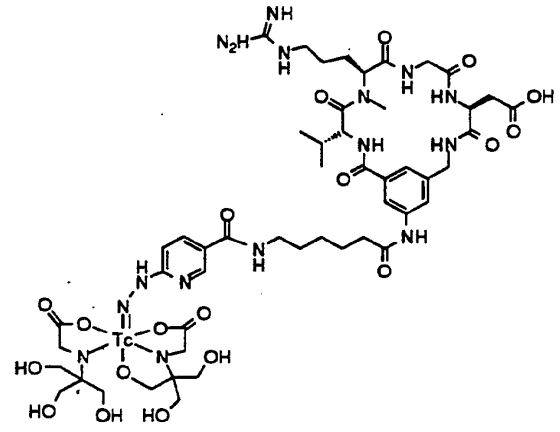

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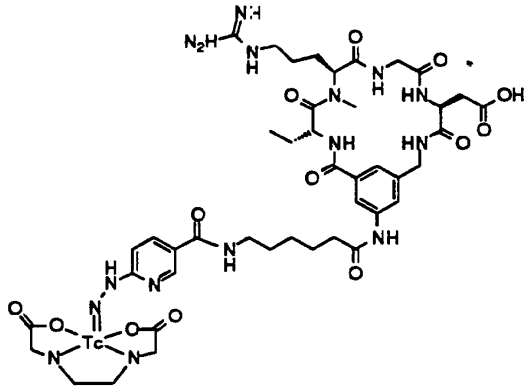

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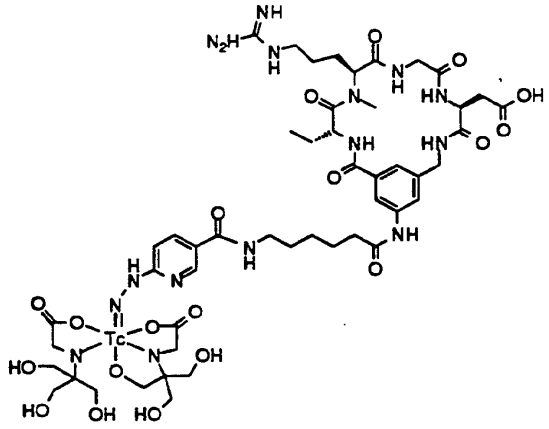

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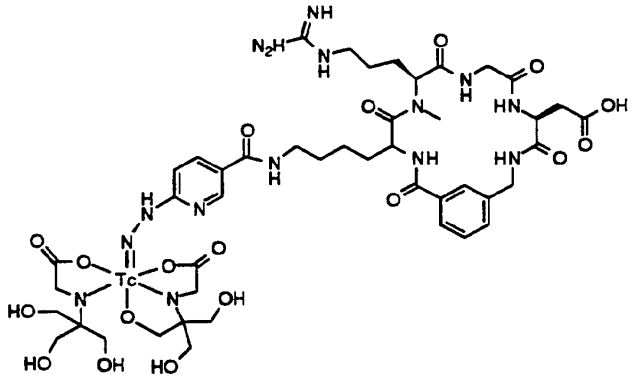

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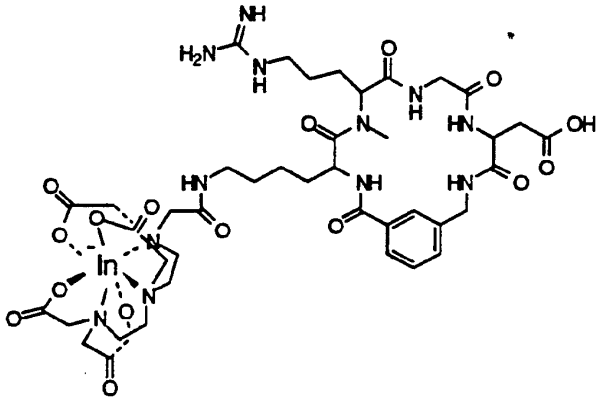

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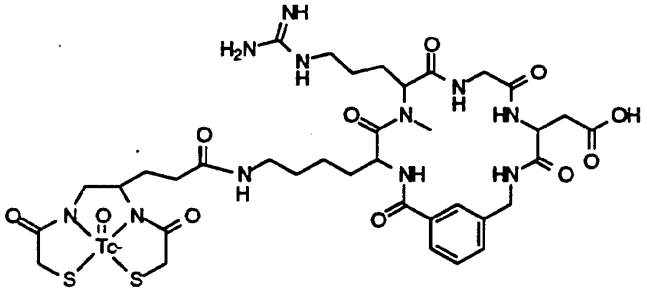

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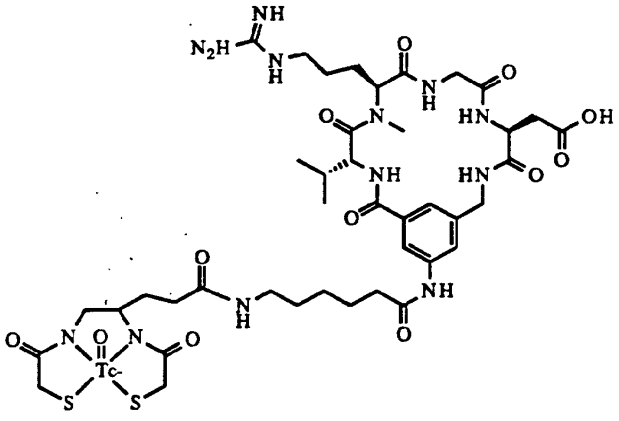

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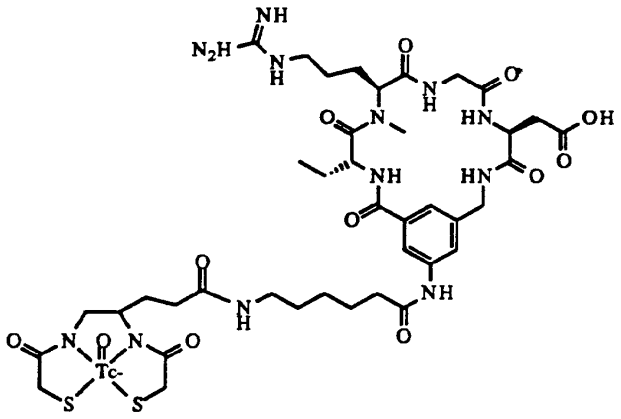

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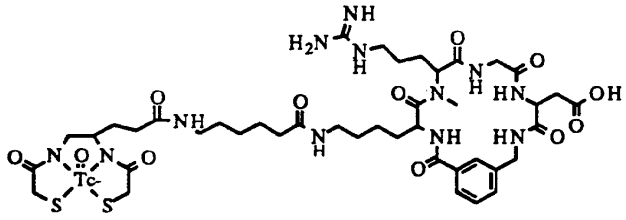

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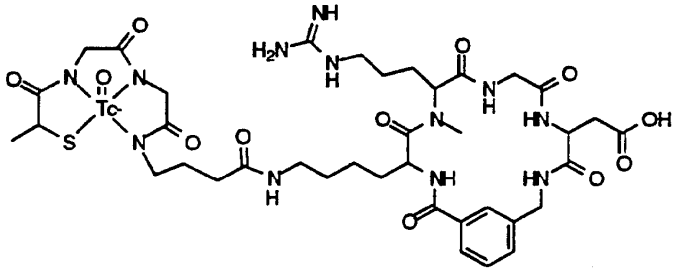

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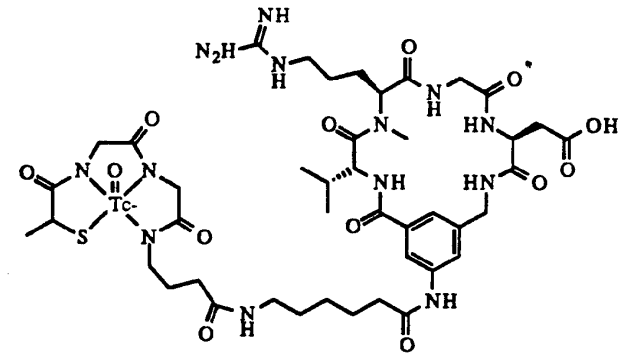

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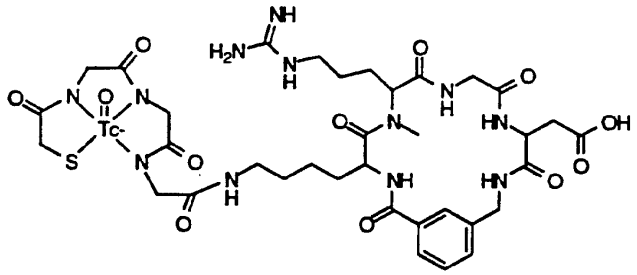

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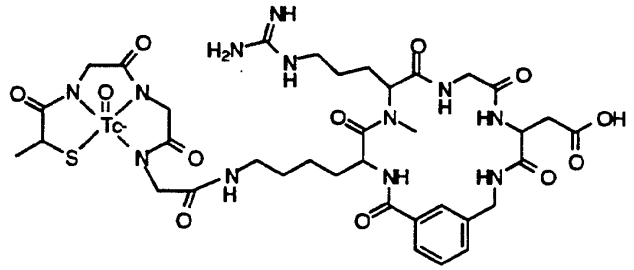

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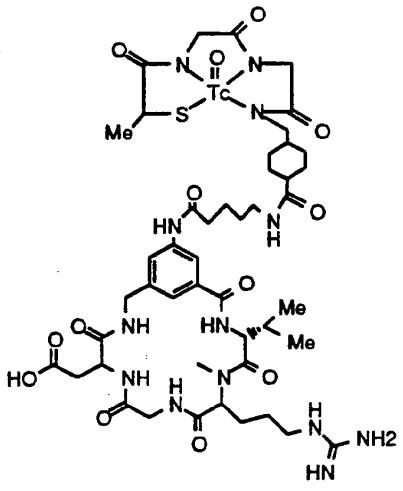

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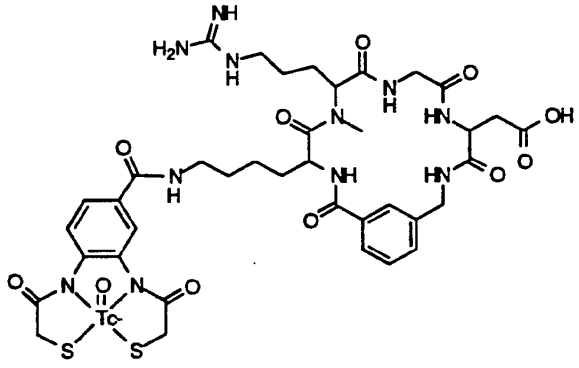

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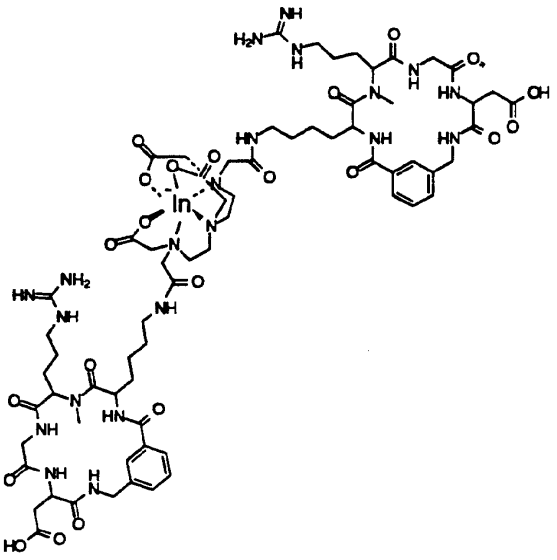


[40] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [29], and (ii) scanning the mammal using a radioimaging devise.

[41] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [30], and (ii) scanning the mammal using a radioimaging devise.

[42] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of

a radiopharmaceutical of [31], and (ii) scanning the mammal using a radioimaging devise.

[43] Also included in the present invention is a method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [32], and (ii) scanning the mammal using a radioimaging devise.

[44] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [33], and (ii) scanning the mammal using a radioimaging devise.

[45] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [34], and (ii) scanning the mammal using a radioimaging devise.

[46] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [35], and (ii) scanning the mammal using a radioimaging devise.

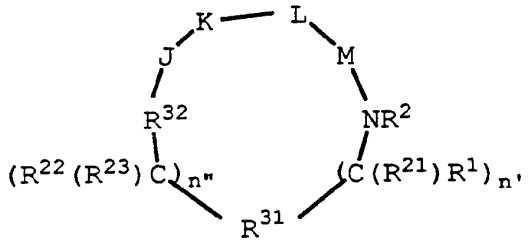
[47] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [36], and (ii) scanning the mammal using a radioimaging devise.

[48] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [37], and (ii) scanning the mammal using a radioimaging devise.

[49] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of [38], and (ii) scanning the mammal using a radioimaging devise.

[50] A method for visualizing sites of platelet deposition in a mammal by radioimaging, comprising (i) administering to said mammal an effective amount of a radiopharmaceutical of Claim 39, and (ii) scanning the mammal using a radioimaging devise.

[51] The present invention is also directed to direct radiolabeled compounds of formula (I):



(I)

or a pharmaceutically acceptable salt or prodrug form thereof wherein:

R^{31} is a C_6-C_{14} saturated, partially saturated, or aromatic carbocyclic ring system substituted with 0-4 R^{10} or R^{10a};

R^{32} is selected from:
-C(=O)-;
-C(=S)-
$-S(=O)_2-$;
-S(=O)-;
$-P(=Z)(ZR^{13})-$;

Z is S or O;

n" and n' are independently 0-2;

R^1 and R^{22} are independently selected from the following groups:

hydrogen,
C_1-C_8 alkyl substituted with 0-2 R^{11};
C_2-C_8 alkenyl substituted with 0-2 R^{11};
C_2-C_8 alkynyl substituted with 0-2 R^{11};
C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, -CN, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, -CHO, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy;

R^1 and R^{21} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n' is 2, R^1 or R^{21} can alternatively be taken together with R^1 or R^{21} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between said carbon atoms;

R^{22} and R^{23} can alternatively join to form a 3-7 membered carbocyclic ring substituted with 0-2 R^{12};

when n" is 2, R^{22} or R^{23} can alternatively be taken together with R^{22} or R^{23} on an adjacent carbon atom to form a direct bond, thereby to form a double or triple bond between the adjacent carbon atoms;

R^1 and R^2, where R^{21} is H, can alternatively join to form a 5-8 membered carbocyclic ring substituted with 0-2 R^{12};

R^{11} is selected from one or more of the following:

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy,

C_1-C_5 alkyl, C_2-C_4 alkenyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_2-C_6 alkoxyalkyl, C_3-C_6 cycloalkoxy, C_1-C_4 alkyl (alkyl being substituted with 1-5 groups selected independently from: $-NR^{13}R^{14}$, $-CF_3$, NO_2, $-SO_2R^{13a}$, or $-S(=O)R^{13a}$),

aryl substituted with 0-2 R^{12},

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, and O, said

heterocyclic ring being substituted with 0-2 R^{12};

R^{12} is selected from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl)$-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, $-(C_1$-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{21} and R^{23} are independently selected from:

hydrogen;
C_1-C_4 alkyl, optionally substituted with 1-6 halogen;
benzyl;

R^2 is H or C_1-C_8 alkyl;

R^{10} and R^{10a} are selected independently from one or more of the following:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)N(R^{13})_2$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy,

ethylenedioxy, C_1-C_4 haloalkyl (including $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)), C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$);

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or C_1-C_8 alkyl;

R^4 is H or C_1-C_3 alkyl;

R^5 is selected from:

hydrogen;

C_1-C_8 alkyl substituted with 0-2 R^{11};

C_2-C_8 alkenyl substituted with 0-2 R^{11};

C_2-C_8 alkynyl substituted with 0-2 R^{11};

C_3-C_{10} cycloalkyl substituted with 0-2 R^{11};

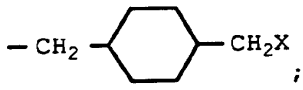
aryl substituted with 0-2 R^{12};

a 5-10-membered heterocyclic ring system containing 1-4 heteroatoms independently selected from N, S, or O, said heterocyclic ring being substituted with 0-2 R^{12};

=O, F, Cl, Br, I, $-CF_3$, $-CN$, $-CO_2R^{13}$, $-C(=O)R^{13}$, $-C(=O)N(R^{13})_2$, $-CHO$, $-CH_2OR^{13}$, $-OC(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{14}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{14}SO_2N(R^{13})_2$, $-NR^{14}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-SR^{13}$, $-S(=O)R^{13a}$, $-SO_2N(R^{13})_2$, $-N(R^{13})_2$, $-NHC(=NH)NHR^{13}$, $-C(=NH)NHR^{13}$, $=NOR^{13}$, NO_2, $-C(=O)NHOR^{13}$, $-C(=O)NHNR^{13}R^{13a}$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, $-SC(=NH)NHR^{13}$, N_3, $-Si(CH_3)_3$, (C_1-C_5 alkyl)NHR^{16};

$-(C_0$-C_6 alkyl)X;

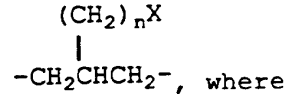

, where q is independently 0,1;

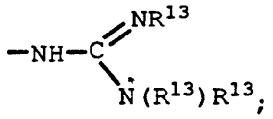

;

$-(CH_2)_mS(O)_{p'}(CH_2)_2X$, where m = 1,2 and p' = 0-2;

wherein X is defined below; and

R^3 and R^4 may also be taken together to form


, where

n = 0,1 and X is  ;

R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ or $-CH_2S(O)_{p'}C(CH_3)_2-$, where t = 2-4 and p' = 0-2; or

R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

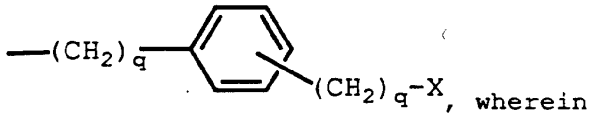
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

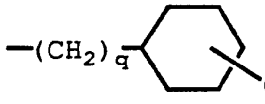
K is a D-isomer or L-isomer amino acid of structure
$-N(R^6)CH(R^7)C(=O)-$, wherein:

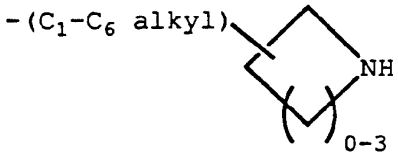
R^6 is H or C_1-C_8 alkyl;

R^7 is selected from:

$-(C_1-C_7$ alkyl)X;

 , wherein
each q is independently 0-2 and substitution on the phenyl is at the 3 or 4 position;

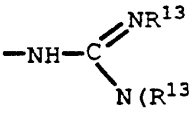

, wherein each q is independently 0-2 and substitution on the cyclohexyl is at the 3 or 4 position;



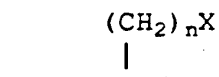
$-(CH_2)_mO-(C_1-C_4$ alkyl)-X, where m = 1 or 2;

$-(CH_2)_mS(O)_{p'}-(C_1-C_4$ alkyl)-X, where m = 1 or 2 and p' = 0-2; and

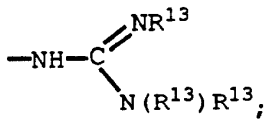
X is selected from:


; $-N(R^{13})R^{13}$; $-C(=NH)(NH_2)$; $-SC(=NH)-NH_2$; $-NH-C(=NH)(NHCN)$; $-NH-C(=NCN)(NH_2)$; $-NH-C(=N-OR^{13})(NH_2)$;

R^6 and R^7 can alternatively be taken together to form


, wherein each q is independently 1 or 2 and wherein

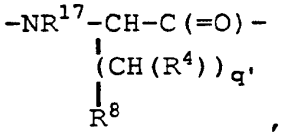
n = 0 or 1 and X is $-NH_2$ or



L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, $N(C_1-C_3$ alkyl), O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl

(said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$;

R^{34} and R^{35} are independently selected from:
-OH,
-F,
$-N(R^{13})_2$, or
C_1-C_8-alkoxy;

R^{34} and R^{35} can alternatively be taken together form:
a cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a divalent cyclic boron amide where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O;
a cyclic boron amide-ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-4 heteroatoms independently selected from N, S, or O; and

wherein the radiolabel is selected from the group: ^{123}I, ^{125}I, ^{131}I, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{75}Br.

[52] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is bonded to $(C(R^{23})R^{22})_{n"}$ and $(C(R^{21})R^{1})_{n'}$ at 2 different atoms on said carbocyclic ring.

[53] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

n" is 0 and n' is 0;
n" is 0 and n' is 1;
n" is 0 and n' is 2;
n" is 1 and n' is 0;
n" is 1 and n' is 1;
n" is 1 and n' is 2;
n" is 2 and n' is 0;
n" is 2 and n' is 1; or
n" is 2 and n' is 2.

[54] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein R^6 is methyl, ethyl, or propyl.

[55] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from the group consisting of:

(a) a 6 membered saturated, partially saturated or aromatic carbocyclic ring substituted with 0-3 R^{10} or R^{10a};

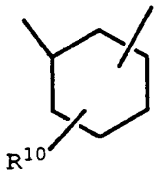
(b) a 8-11 membered saturated, partially saturated, or aromatic fused bicyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}; or

(c) a 14 membered saturated, partially saturated, or aromatic fused tricyclic carbocyclic ring substituted with 0-4 R^{10} or R^{10a}.

[56] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from the group consisting of:

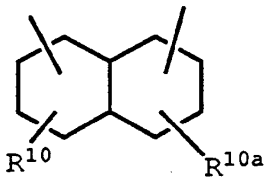
(a) a 6 membered saturated, partially saturated, or aromatic carbocyclic ring of formula:



wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10};

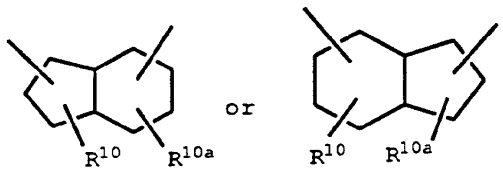
(b) a 10 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



, wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a};

(c) a 9 membered saturated, partially saturated, or aromatic bicyclic carbocyclic ring of formula:



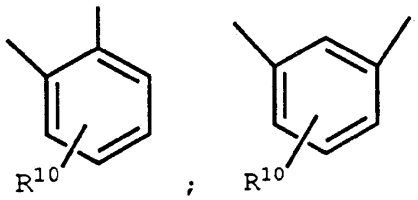
,

wherein any of the bonds forming the carbocyclic ring may be a single or double bond,

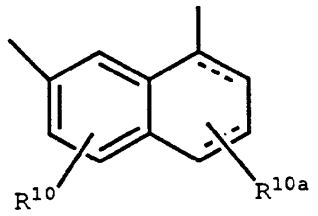
and wherein said carbocyclic ring is substituted independently with 0-4 R^{10} or R^{10a}.

[57] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from (the dashed bond may be a single or double bond):



; ; ; or



;

n" is 0 or 1; and

n' is 0-2.

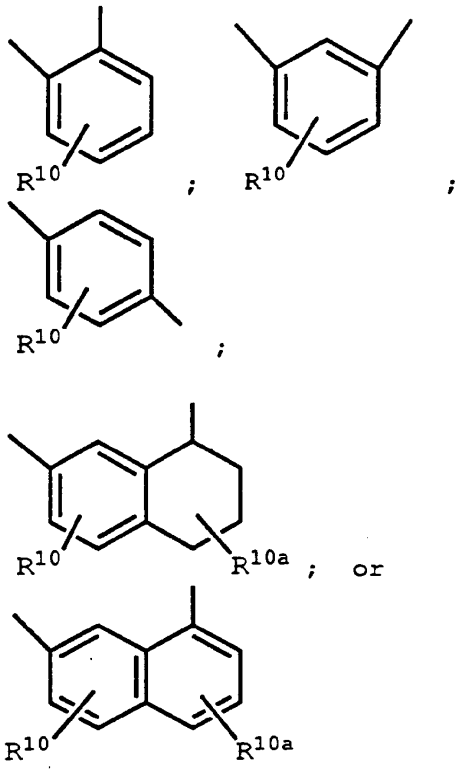
[58] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^1 and R^{22} are independently selected from:

phenyl, benzyl, phenethyl, phenoxy, benzyloxy, halogen, hydroxy, nitro, cyano, C_1-C_5 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_7-C_{10} arylalkyl, C_1-C_5 alkoxy, $-CO_2R^{13}$, $-C(=O)NHOR^{13a}$, $-C(=O)NHN(R^{13})_2$, $=NOR^{13}$, $-B(R^{34})(R^{35})$, C_3-C_6 cycloalkoxy, $-OC(=O)R^{13}$, $-C(=O)R^{13}$, $-OC(=O)OR^{13a}$, $-OR^{13}$, $-(C_1$-C_4 alkyl$)-OR^{13}$, $-N(R^{13})_2$, $-OC(=O)N(R^{13})_2$, $-NR^{13}C(=O)R^{13}$, $-NR^{13}C(=O)OR^{13a}$, $-NR^{13}C(=O)N(R^{13})_2$, $-NR^{13}SO_2N(R^{13})_2$, $-NR^{13}SO_2R^{13a}$, $-SO_3H$, $-SO_2R^{13a}$, $-S(=O)R^{13a}$, $-SR^{13}$, $-SO_2N(R^{13})_2$, C_2-C_6 alkoxyalkyl, methylenedioxy, ethylenedioxy, C_1-C_4 haloalkyl, C_1-C_4 haloalkoxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkylcarbonyl, C_1-C_4 alkylcarbonylamino, $-OCH_2CO_2H$, 2-(1-morpholino)ethoxy, C_1-C_4 alkyl (alkyl being substituted with $-N(R^{13})_2$, $-CF_3$, NO_2, or $-S(=O)R^{13a}$).

[59] Included in the present invention are those direct radiolabeled compounds in [51] above, wherein:

R^{31} is selected from:



; ; ; or

wherein R^{31} may be substituted independently with 0-3 R^{10} or R^{10a};

R^{32} is -C(=O)-;

n" is 0 or 1;

n' is 0-2;

R^1 and R^{22} are independently selected from H, C_1-C_4 alkyl, phenyl, benzyl, phenyl-(C_2-C_4)alkyl, C_1-C_4 alkoxy;

R^{21} and R^{23} are independently H or C_1-C_4 alkyl;

R^2 is H or C_1-C_8 alkyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

R^{10} and R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3,

$CH_2CH_2SCH_3$, $(CH_2)_sNH_2$,
$-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where
s = 3-5; or

R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

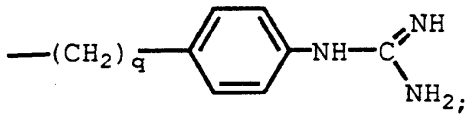
R^3 and R^5 can alternatively be taken together to form $-(CH_2)_t-$ (t = 2-4) or $-CH_2SC(CH_3)_2-$; or

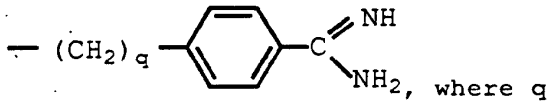
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

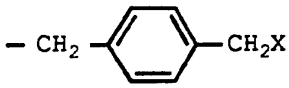
R^7 is





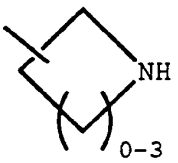
where q = 0 or 1;
$-(CH_2)_rX$, where r = 3-6;


;


;

$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)

$-(C_1-C_4$ alkyl) 

$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$; or

R^6 and R^7 can alternatively be taken together to form

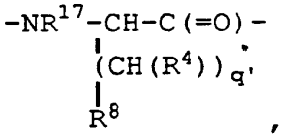
$-CH_2CH(CH_2)_nX-CH_2-$, where n = 0 or 1

and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1 or 2;

M is a D-isomer or L-isomer amino acid of structure



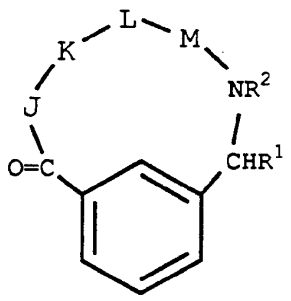
wherein:

q' is 0-2;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:
$-CO_2R^{13}$,$-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O), $-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$, $-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$, $-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[60] Included in the present invention are those direct radiolabeled compounds in [51] above, that are radiolabeled 1,3-disubstituted phenyl compounds of the formula (II):



(II)

wherein:

the shown phenyl ring in formula (II) may be further substituted with 0-3 R^{10};

R^{10} is selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_1-C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form -$(CH_2)_{2-5}$- or -$(CH_2)O(CH_2)$-;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H or C_1-C_3 alkyl;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $-(CH_2)_sNHC(=NH)(NH_2)$, $-(CH_2)_sNHR^{16}$, where s = 3-5; or

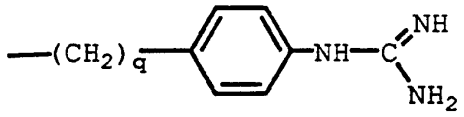
R^{16} is selected from:
an amine protecting group;
1-2 amino acids; or
1-2 amino acids substituted with an amine protecting group;

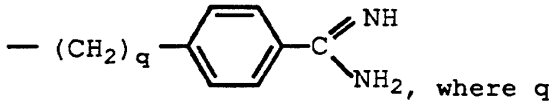
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$; or
R^4 and R^5 can alternatively be taken together to form $-(CH_2)_u-$, where u = 2-5;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_1-C_8 alkyl;

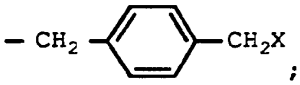
R^7 is:





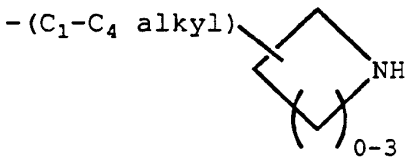
= 0 or 1;

$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_3-C_7$ alkyl)$-NH-(C_1-C_6$ alkyl)



$-(CH_2)_m-O-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2;

$-(CH_2)_m-S-(C_1-C_4$ alkyl)$-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

R^6 and R7 are alternatively be taken together to form

$-CH_2CH(CH_2)_nXCH_2-$, where n = 0,1 and X is $-NH_2$ or $-NHC(=NH)(NH_2)$;

L is $-Y(CH_2)_vC(=O)-$, wherein:

Y is NH, O, or S; and v = 1,2;

M is a D-isomer or L-isomer amino acid of structure

$$-NR^{17}-CH(CH(R^4))_{q'}-C(=O)-$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad\quad R^8$$

wherein:

q' is 0-2;

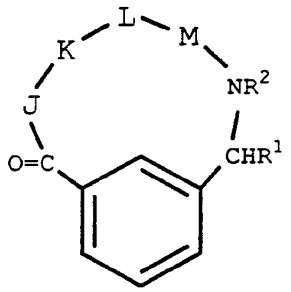
R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2R^{13}$, $-SO_3R^{13}$, $-SO_2NHR^{14}$, $-B(R^{34})(R^{35})$, $-NHSO_2CF_3$, $-CONHNHSO_2CF_3$, $-PO(OR^{13})_2$, $-PO(OR^{13})R^{13}$, $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected independently from N, S, or O) , $-SO_2NH$-heteroaryl (said heteroaryl being 5-10-membered and having 1-4 heteroatoms selected

independently from N, S, or O),
$-SO_2NHCOR^{13}$, $-CONHSO_2R^{13a}$,
$-CH_2CONHSO_2R^{13a}$, $-NHSO_2NHCOR^{13a}$,
$-NHCONHSO_2R^{13a}$, $-SO_2NHCONHR^{13}$.

[61] Included in the present invention are those direct radiolabeled compounds in [51] above, that are radiolabeled 1,3-disubstituted phenyl compounds of the formula (II):



(II)

wherein:

the phenyl ring in formula (II) may be further substituted with 0-3 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H, C_1-C_4 alkyl, phenyl, benzyl, or phenyl-(C_2- C_4)alkyl;

R^2 is H or methyl;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12}

alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of structure $-N(R^3)C(R^4)(R^5)C(=O)-$, wherein:

R^3 is H or CH_3;

R^4 is H;

R^5 is H, C_1-C_8 alkyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylmethyl, C_1-C_6 cycloalkylethyl, phenyl, phenylmethyl, CH_2OH, CH_2SH, CH_2OCH_3, CH_2SCH_3, $CH_2CH_2SCH_3$, $(CH_2)_sNH_2$, $(CH_2)_sNHC(=NH)(NH_2)$, $(CH_2)_sR^{16}$, where s = 3-5;

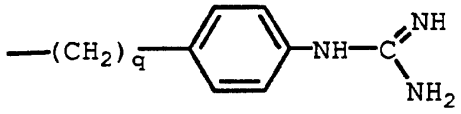
R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

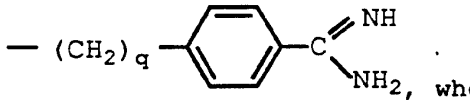
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of structure $-N(R^6)CH(R^7)C(=O)-$, wherein:

R^6 is H or C_3-C_8 alkyl;

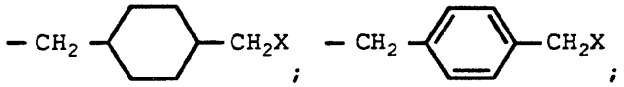
R^7 is





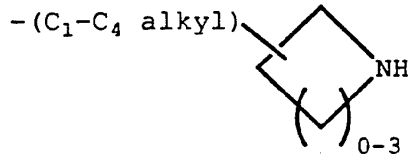
where q = 0 or 1;

$-(CH_2)_rX$, where r = 3-6;



$-(CH_2)_mS(CH_2)_2X$, where m = 1 or 2;

$-(C_4$-C_7 alkyl$)$-NH-$(C_1$-C_6 alkyl$)$



$-(CH_2)_m$-O-$(C_1$-C_4 alkyl$)$-NH-$(C_1$-C_6 alkyl), where m = 1 or 2;

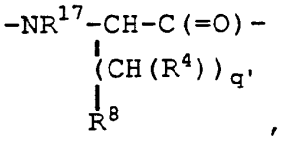
$-(CH_2)_m-S-(C_1-C_4$ alkyl$)-NH-(C_1-C_6$ alkyl), where m = 1 or 2; and

X is $-NH_2$ or $-NHC(=NH)(NH_2)$, provided that X is not $-NH_2$ when r = 4; or

L is $-YCH_2C(=O)-$, wherein:

Y is NH or O;

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^{17} is H, C_1-C_3 alkyl;

R^8 is selected from:

$-CO_2H$ or $-SO_3R^{13}$.

[62] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

the phenyl ring in formula (II) may be further substituted with 0-2 R^{10} or R^{10a};

R^{10} or R^{10a} are selected independently from: H, C_1-C_8 alkyl, phenyl, halogen, or C_1-C_4 alkoxy;

R^1 is H;

R^2 is H;

R^{13} is selected independently from: H, C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

R^{13a} is C_1-C_{10} alkyl, C_3-C_{10} cycloalkyl, C_4-C_{12} alkylcycloalkyl, aryl, -(C_1-C_{10} alkyl)aryl, or C_3-C_{10} alkoxyalkyl;

when two R^{13} groups are bonded to a single N, said R^{13} groups may alternatively be taken together to form $-(CH_2)_{2-5}-$ or $-(CH_2)O(CH_2)-$;

R^{14} is OH, H, C_1-C_4 alkyl, or benzyl;

J is β-Ala or an L-isomer or D-isomer amino acid of formula $-N(R^3)CH(R^5)C(=O)-$, wherein:

R^3 is H and R^5 is H, CH_3, CH_2CH_3, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$, $CH_2CH_2CH_3$, $CH_2CH_2CH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH(CH_3)_2$, $(CH_2)_4NH_2$, (C_3-C_5 alkyl)NHR^{16};
or

R^3 is CH_3 and R^5 is H; or

R^3 and R^5 can alternatively be taken together to form $-CH_2CH_2CH_2-$;

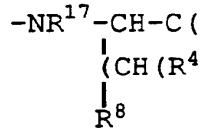
R^{16} is selected from:
an amine protecting group;
1-2 amino acids;
1-2 amino acids substituted with an amine protecting group;

K is an L-isomer amino acid of formula $-N(CH_3)CH(R^7)C(=O)-$, wherein:

R^7 is $-(CH_2)_3NHC(=NH)(NH_2)$;

L is $-NHCH_2C(=O)-$; and

M is a D-isomer or L-isomer amino acid of structure



, wherein:

q' is 1;

R^4 is H or CH_3;

R^{17} is H;

R^8 is
$-CO_2H$;
$-SO_3H$.

[63] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala, N^{ε}-p-azidobenzoyl-D-Lys, N^{ε}-p-benzoylbenzoyl-D-Lys, N^{ε}-tryptophanyl-D-Lys, N^{ε}-o-benzylbenzoyl-D-Lys, N^{ε}-p-acetylbenzoyl-D-Lys, N^{ε}-dansyl-D-Lys, N^{ε}-glycyl-D-Lys, N^{ε}-glycyl-p-benzoylbenzoyl-D-Lys, N^{ε}-p-phenylbenzoyl-D-Lys, N^{ε}-m-benzoylbenzoyl-D-Lys, N^{ε}-o-benzoylbenzoyl-D-Lys;

K is selected from NMeArg, Arg;

L is selected from Gly, β-Ala, Ala;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[64] Included in the present invention are those direct radiolabeled compounds in of formula (II) above, wherein:

R^1 and R^2 are independently selected from H, methyl;

J is selected from: D-Val, D-2-aminobutyric acid, D-Leu, D-Ala, Gly, D-Pro, D-Ser, D-Lys, β-Ala, Pro, Phe, NMeGly, D-Nle, D-Phg, D-Ile, D-Phe, D-Tyr, Ala;

K is selected from NMeArg;

L is Gly;

M is selected from Asp; αMeAsp; βMeAsp; NMeAsp; D-Asp.

[65] Included in the present invention are those direct radiolabeled compounds of [51] that are:

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-2-aminobutyric acid; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Leu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is Gly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Pro; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Lys; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is β-Ala; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is NMeGly; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is methyl (isomer 2); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 is phenyl (isomer 1); R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein J = D-Met, K = NMeArg, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-guanidinyl-Orn , L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein J = D-Abu, K = diNMe-Lys, L = Gly, M = Asp, R^1 = H, R^2 = H;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-azidobenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-tryptophanyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-acetylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-dansyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

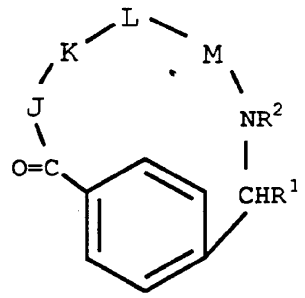
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-glycyl-p-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-p-phenylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-m-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is N^{ε}-o-benzoylbenzoyl-D-Lysine; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(III);

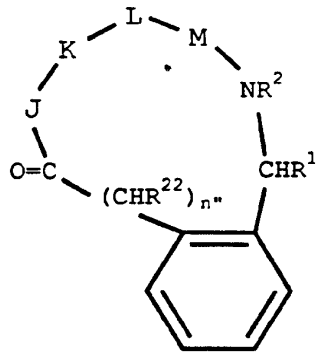
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is D-NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Nle; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phg; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Phe; K is NMeArg; L is Gly; and M is Asp;

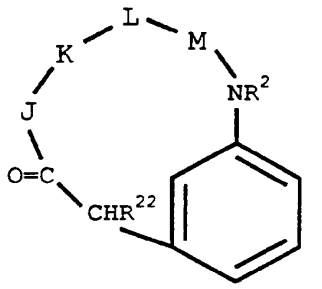
the radiolabeled compound of formula (V) wherein R^1 and R^2 are H; J is D-Ile; K is NMeArg; L is Gly; and M is Asp;



(V);

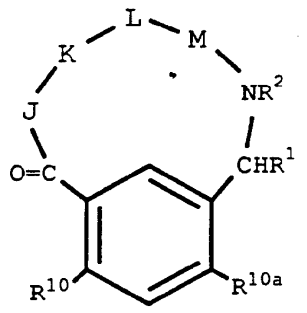
the radiolabeled compound of formula (V) wherein n"=1; R^1, R^2, and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (V) wherein n"=0; R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VI)

the radiolabeled compound of formula (VI) wherein R^2 and R^{22} are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;



(VII)

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Cl; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is MeO; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10a} are H; R^{10} is Me; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1,R^2, and R^{10} are H; R^{10a} is Cl; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is I; J is D-Abu; K is NMeArg; L is Gly; and M is Asp.

The radiolabeled compound of formula (VII) wherein R^1, R^2, and R^{10} are H; R^{10a} is Me; J is D-Abu; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Tyr; K is NMeArg; L is Gly; and M is Asp;

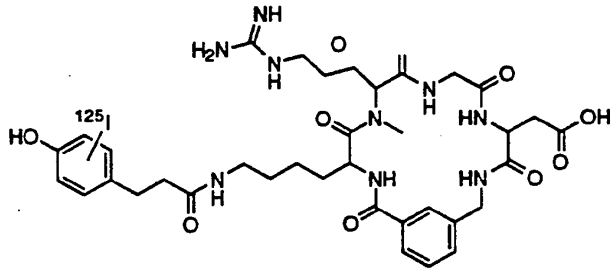
the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeAmf; L is Gly; and M is Asp;

the radiolabeled compound of formula (II) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is βMeAsp;

the radiolabeled compound of formula (II) wherein R^1 is H; R^2 is CH_3; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (III) wherein R^1 and R^2 are H; J is D-Val; K is NMeArg; L is Gly; and M is Asp;

the radiolabeled compound of formula (VIII) wherein J is D-Val; K is NMeArg; L is Gly; and M is Asp;



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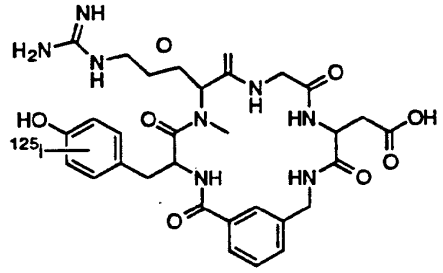

[66] Included in the present invention are those radiolabeled compound as in one of [51]-[65] wherein the radiolabel is selected from the group: ^{18}F, ^{11}C, ^{123}I, and ^{125}I.

[67] Included in the present invention are those radiolabeled compounds of [66] wherein the radiolabel is ^{123}I.

[68] Included in the present invention is a radiopharmaceutical composition comprising a radiopharmaceutically acceptable carrier and a radiolabeled compound of any of [51]-[67].

[69] Included in the present invention is a method of determining platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of [51]-[67], and imaging said mammal.

[70] Included in the present invention is a method of diagnosing a disorder associated with platelet deposition in a mammal comprising administering to said mammal a radiopharmaceutical composition comprising a compound of any of [51]-[67], and imaging said mammal.

As noted above, the cyclic compounds of the present invention are radiolabeled. By "radiolabeled", it is meant that the subject cyclic platelet glycoprotein IIb/IIIa compounds contain a radioisotope which is suitable for administration to a mammalian patient. Suitable radioisotopes are known to those skilled in the art and include, for example, isotopes of halogens (such as chlorine, fluorine, bromine and iodine), and metals including technetium and indium. Preferred radioisotopes include 11C, 18F, ^{123}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{94m}Tc, ^{95}Tc, ^{111}In, ^{62}Cu, ^{43}Sc, ^{45}Ti, ^{67}Ga, ^{68}Ga, ^{97}Ru, ^{72}As, ^{82}Rb, and ^{201}Tl. Most preferred are the isoptopes ^{123}I, ^{111}In, and ^{99m}Tc. Radiolabeled compounds of the invention may be prepared using standard radiolabeling procedures well known to those skilled in the art.

Suitable synthesis methodology is described in detail below. As discussed below, the cyclic platelet glycoprotein IIb/IIIa compounds of the invention may be radiolabeled either directly (that is, by incorporating the radiolabel directly into the compounds) or indirectly (that is, by incorporating the radiolabel into the compounds through a chelating agent, where the chelating agent has been incorporated into the compounds). Also, the radiolabeling may be isotopic or nonisotopic. With isotopic radiolabeling, one group already present in the cyclic compounds described above is substituted with (exchanged for) the radioisotope. With nonisotopic radiolabeling, the radioisotope is added to the cyclic compounds without substituting with (exchanging for) an already existing group. Direct and indirect radiolabeled compounds, as well as isotopic and nonisotopic radiolabeled compounds are included within the phrase "radiolabeled compounds" as used in connection with the present invention. Such radiolabeling should also be reasonably stable, both chemically and metabolically, applying recognized standards in the art. Also, although the compounds of the invention may be labeled in a variety of fashions with a variety of different radioisotopes, as those skilled in the art will recognize, such radiolabeling should be carried out in a manner such that the high binding affinity and specificity of the unlabeled cyclic platelet GPIIb/IIIa compounds of the invention to the GPIIb/IIIa receptor is not significantly affected. By not significantly affected, it is meant that the binding affinity and specificity is not affected more than about 3 log units, preferably not more than about 2 log units, more preferably not more than about 1 log unit, even more preferably not more than about 500%, and still even

more preferably not more than about 250%, and most preferably the binding affinity and specificity is not affected at all.

For radiolabeled compounds, the label may appear at any position on Q. Preferred radiolabeled compounds of the invention are radiolabeled compounds wherein the radiolabel is located on the carbocyclic ring system of R^{31}, the R^5 substituent on J, and at R^1 or R^{22}. Even more preferred radiolabeled compounds of the invention are those of formula (II), wherein the radiolabel is located on the carbocyclic ring system of R^{31}, or the R^5 substituent on J. With regard to the preferred and more preferred direct radiolabeled compounds, the preferred radiolabel is a halogen label, especially an iodine radiolabel. For indirect radiolabeled compounds, the preferred metal nuclides are ^{99m}Tc and ^{111}In. Preferred linking groups, Ln, and metal chelators, C_h, are described below.

It has been discovered that the radiolabeled compounds of the invention are useful as radiopharmaceuticals for non-invasive imaging to diagnose present or potential thromboembolic disorders, such as arterial or venous thrombosis, including, for example, unstable angina, myocardial infarction, transient ischemic attack, stroke, atherosclerosis, diabetes, thrombophlebitis, pulmonary emboli, or platelet plugs, thrombi or emboli caused by prosthetic cardiac devices such as heart valves. The radiolabeled compounds of the invention are useful with both newly formed and older thrombi. The radiolabeled compounds of the invention may also be used to diagnose other present or potential conditions where there is overexpression of the GPIIb/IIIa receptors, such as with metastatic cancer cells. The subject compounds may be effectively

employed in low doses, thereby minimizing any risk of toxicity. Also, the subject compounds are of a much smaller size than, for example, the radiolabeled 7E3 antibodies known in the art, allowing easier attainment of suitable target/background (T/B) ratio for detecting thrombi. The use of the radiolabeled compounds of the invention is further described in the utility section below.

In the present invention it has also been discovered that the radiolabeled compounds above are useful as inhibitors of glycoprotein IIb/IIIa (GPIIb/IIIa), and thus the radiolabeled compounds of the invention may also be employed for therapeutic purposes, in addition to the diagnostic usage described above. As discussed above, GPIIb/IIIa mediates the process of platelet activation and aggregation. The radiolabeled compounds of the present invention inhibit the activation and aggregation of platelets induced by all known endogenous platelet agonists.

The compounds herein described may have asymmetric centers. Unless otherwise indicated, all chiral, diastereomeric and racemic forms are included in the present invention. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. It will be appreciated that compounds of the present invention contain asymmetrically substituted carbon atoms, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. Two distinct isomers (cis and trans) of the peptide bond are known to occur; both can

also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Unless otherwise specifically noted, the L-isomer of the amino acid is used at positions J, K, L, and M of the compounds of the present invention. Except as provided in the preceding sentence, all chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated. The D and L-isomers of a particular amino acid are designated herein using the conventional 3-letter abbreviation of the amino acid, as indicated by the following examples: D-Leu, D-Leu, L-Leu, or L-Leu.

When any variable (for example, R^1 through R^8, m, n, p, X, Y, etc.) occurs more than one time in any constituent or in any formula, its definition on each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R^{11}, then said group may optionally be substituted with up to two R^{11} and R^{11} at each occurrence is selected independently from the defined list of possible R^{11}. Also, by way of example, for the group $-N(R^{13})_2$, each of the two R^{13} substituents on N is independently selected from the defined list of possible R^{13}.

When a bond to a substituent is shown to cross the bond connecting two atoms in a ring, then such substituent may be bonded to any atom on the ring.

Combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction