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.

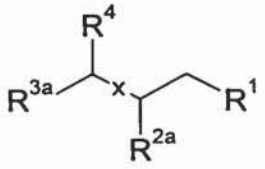
2. A compound as claimed in claim 1 wherein:

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,

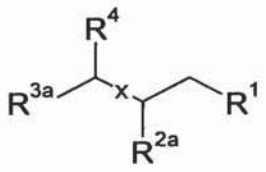
or x-$R^{3a}R^4$ together represent hydrogen in which case R^1 is $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$,

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.

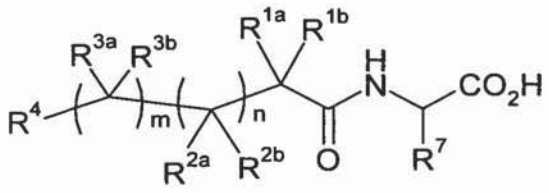
7. The compound of claim 6, wherein R^{3a} represents a substituted or nonsubstituted aryl group.

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.

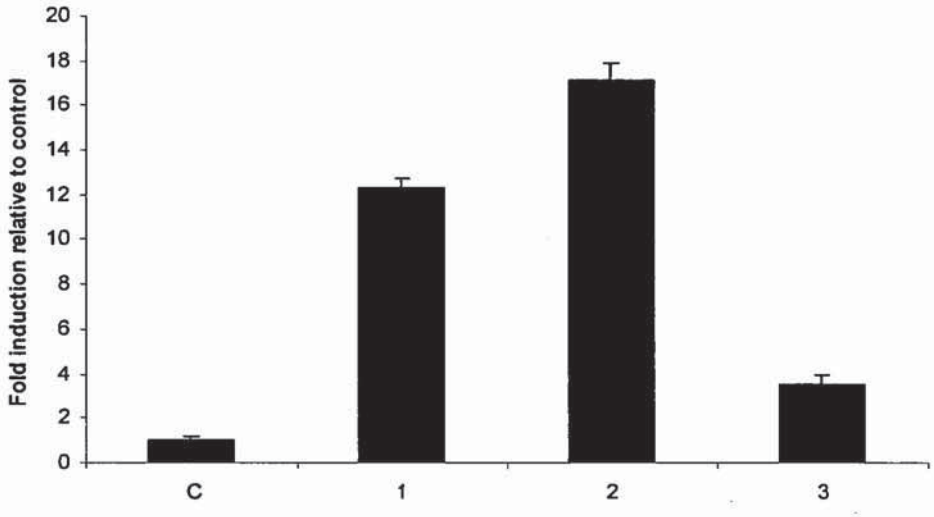

Fold induction relative to control
20
18
16
14
12
10
8
6
4
2
0
C
1
2
3

Figure 1.

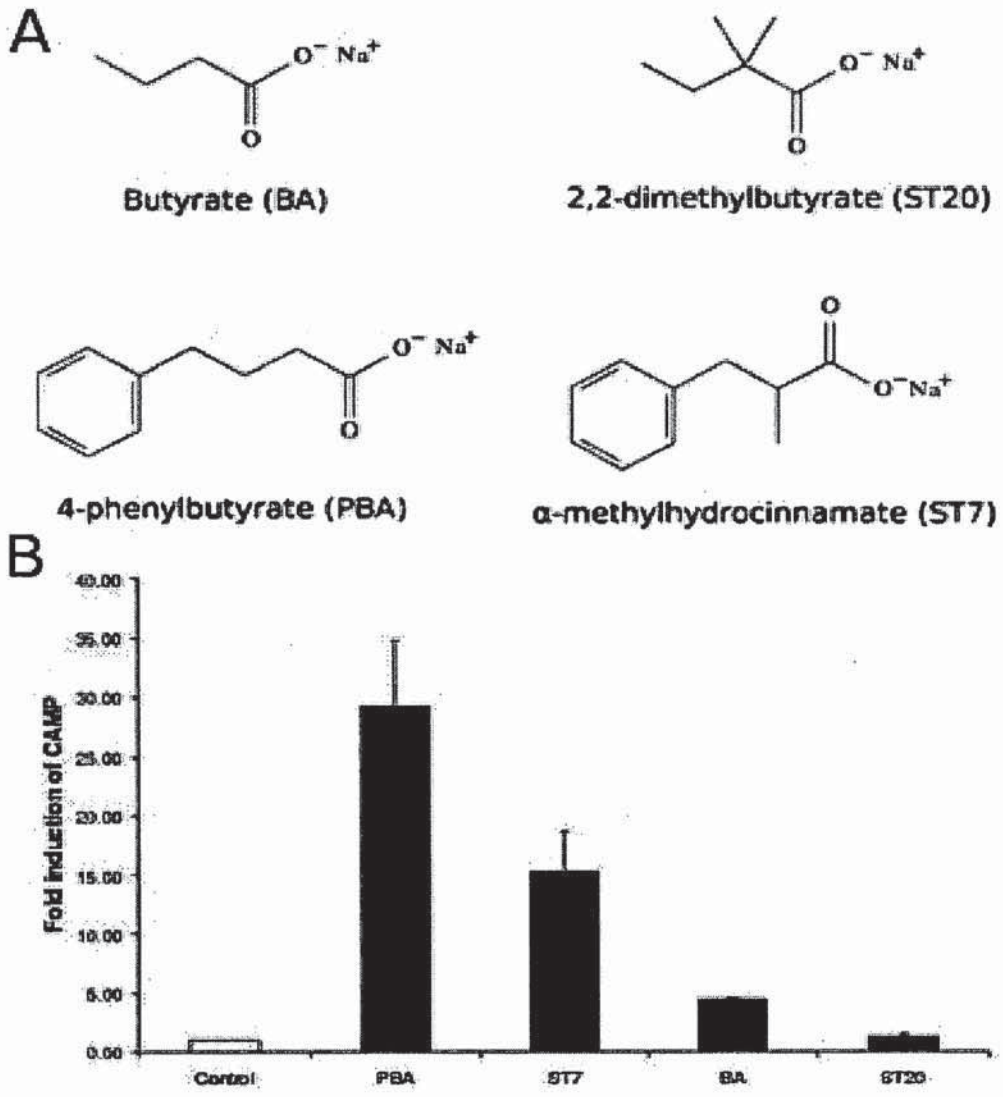

A
Butyrate (BA)
2,2-dimethylbutyrate (ST20)
4-phenylbutyrate (PBA)
α-methylhydrocinnamate (ST7)
B
Fold induction of CAMP
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Control
PBA
ST7
BA
ST20

Figure 2

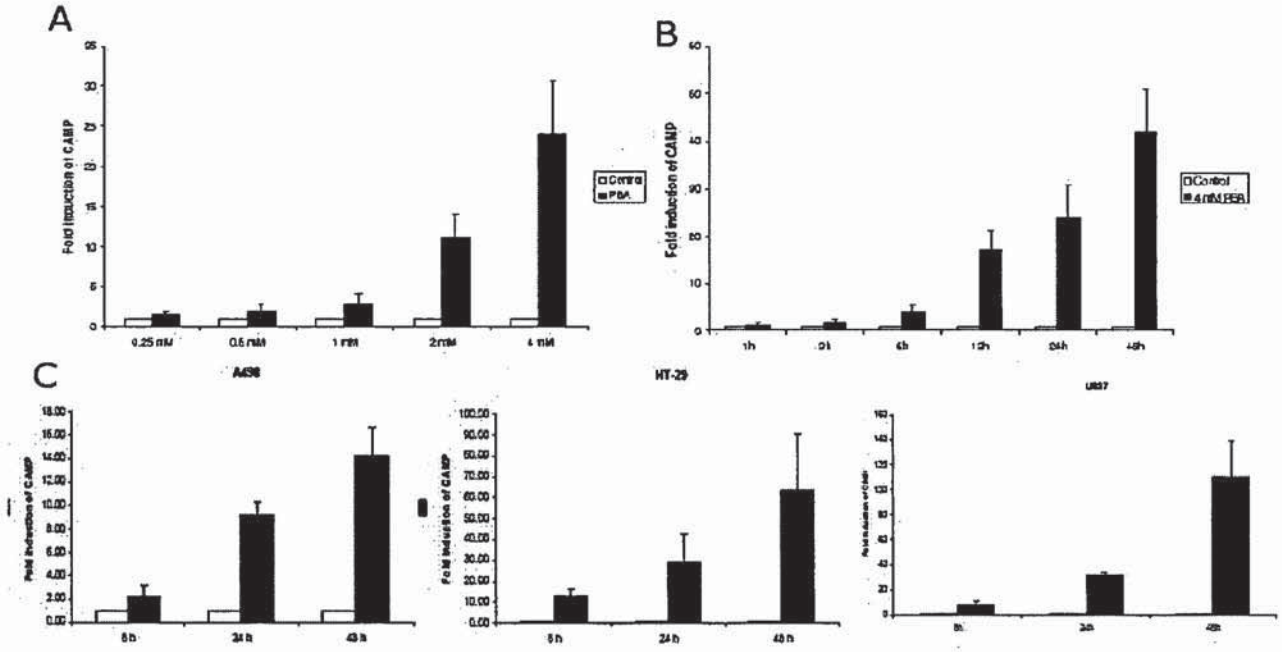


Figure 3

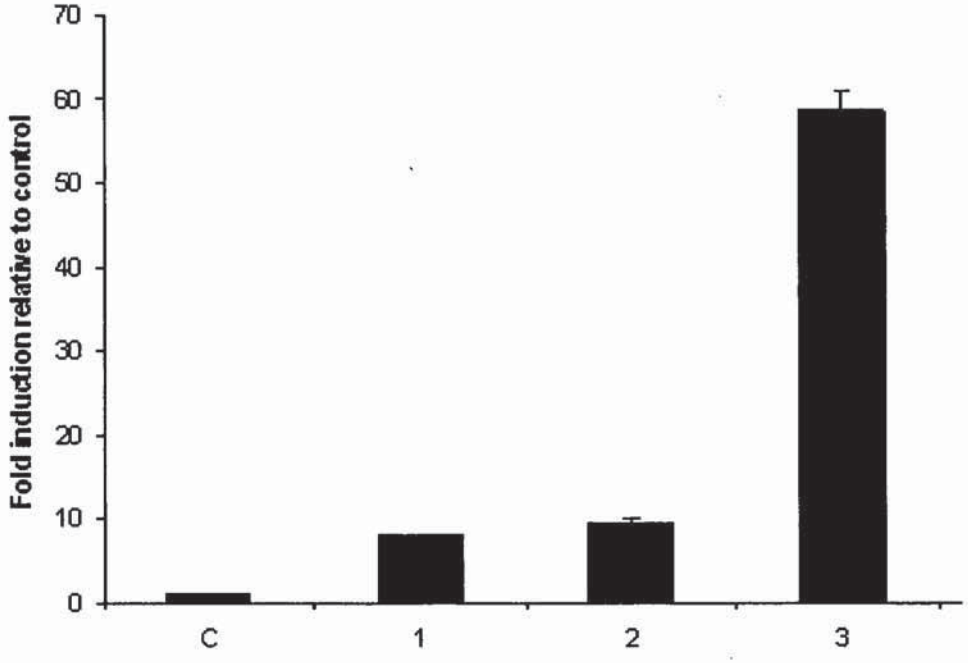


Figure 4

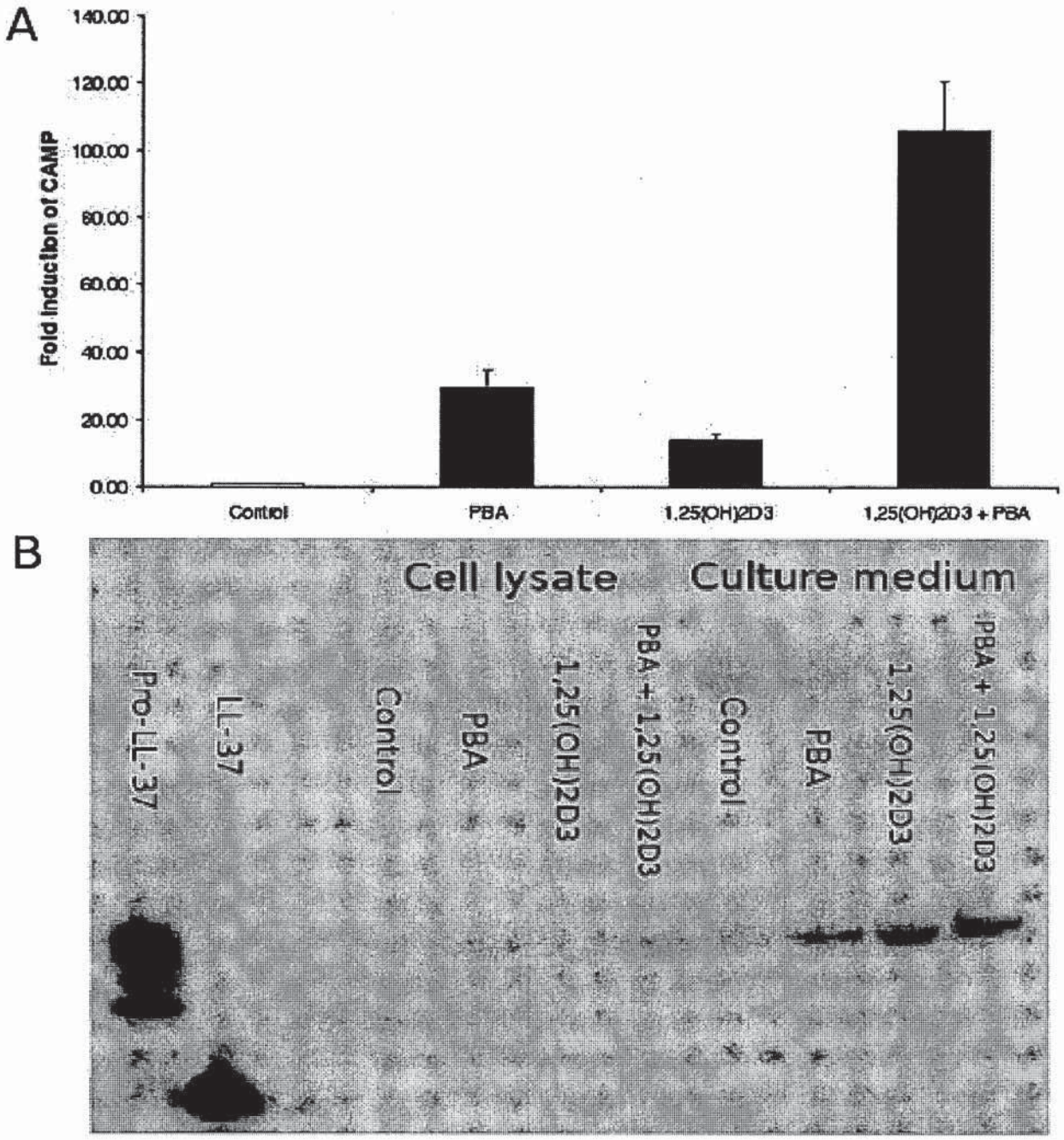

A
Fold induction of CAMP
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Control
PBA
1,25(OH)2D3
1,25(OH)2D3 + PBA
B
Cell lysate
Culture medium
Pro-LL-37
LL-37
Control
PBA
1,25(OH)2D3
PBA + 1,25(OH)2D3
Control
PBA
1,25(OH)2D3
PBA + 1,25(OH)2D3

Figure 5

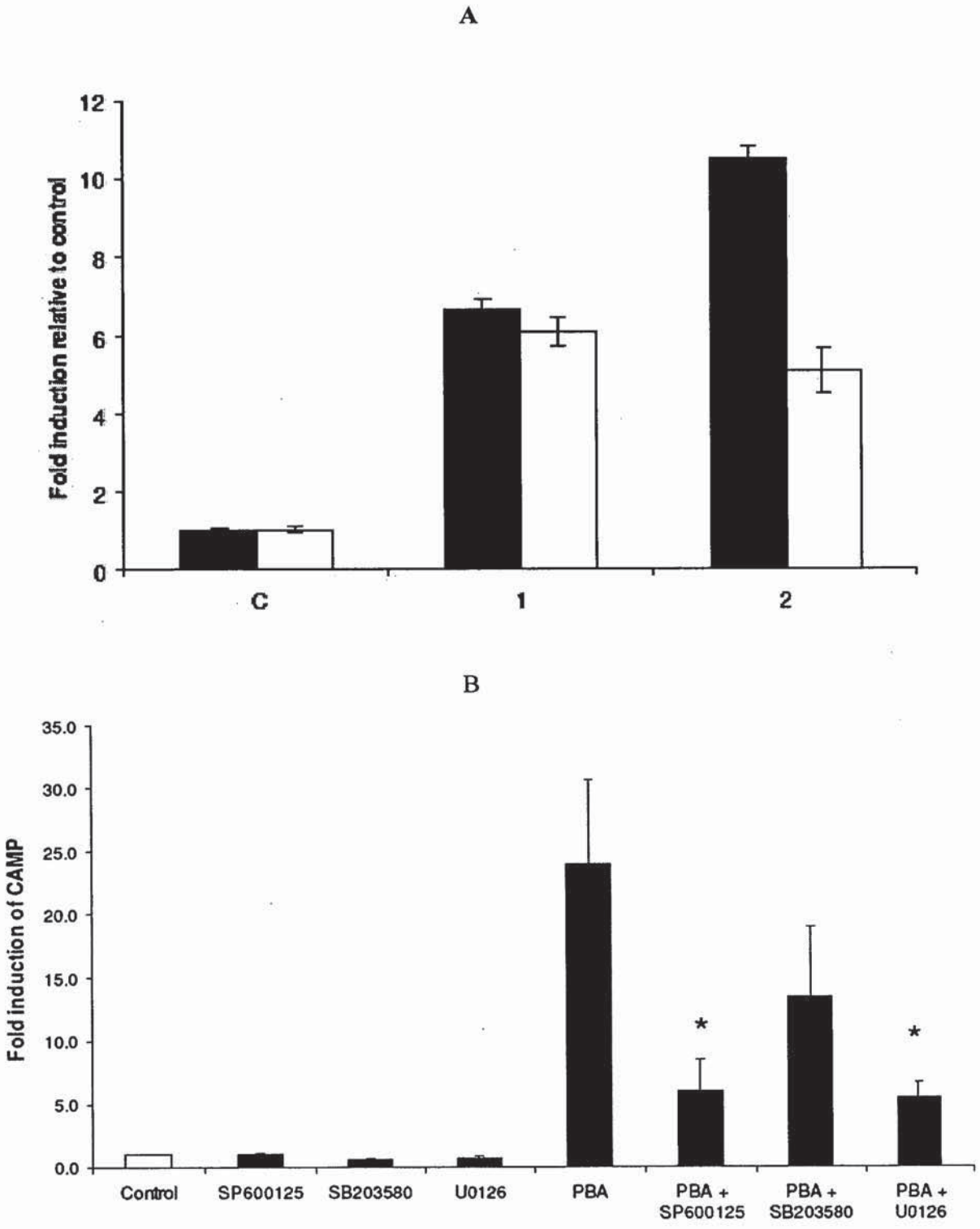

A
Fold induction relative to control
12
10
8
6
4
2
0
C
1
2
B
Fold induction of CAMP
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
*
*
Control
SP600125
SB203580
U0126
PBA
PBA + SP600125
PBA + SB203580
PBA + U0126

Figure 6

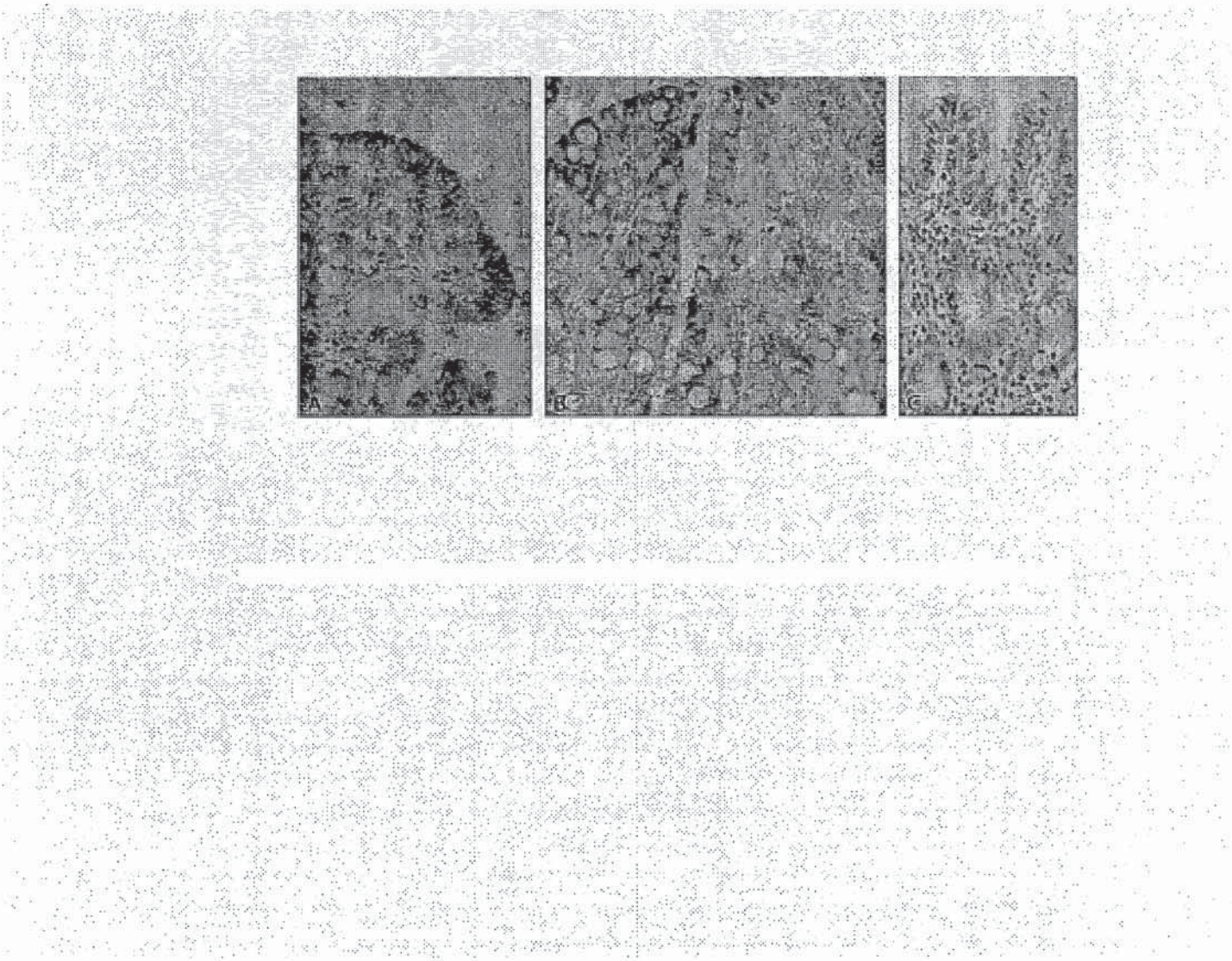


Figure 7

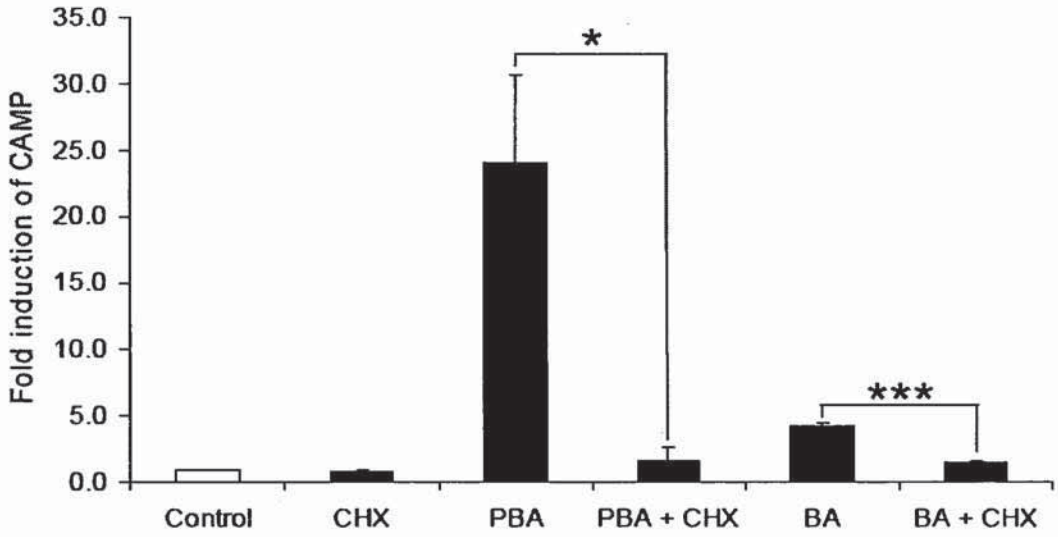


Figure 8

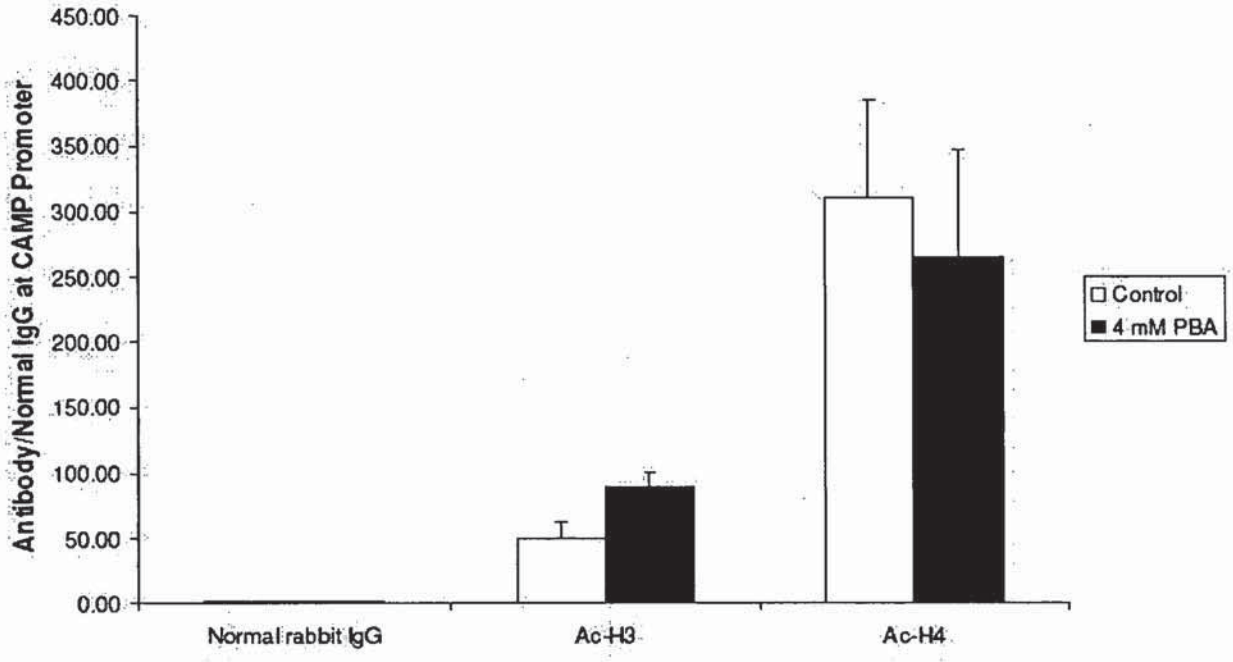


Figure 9

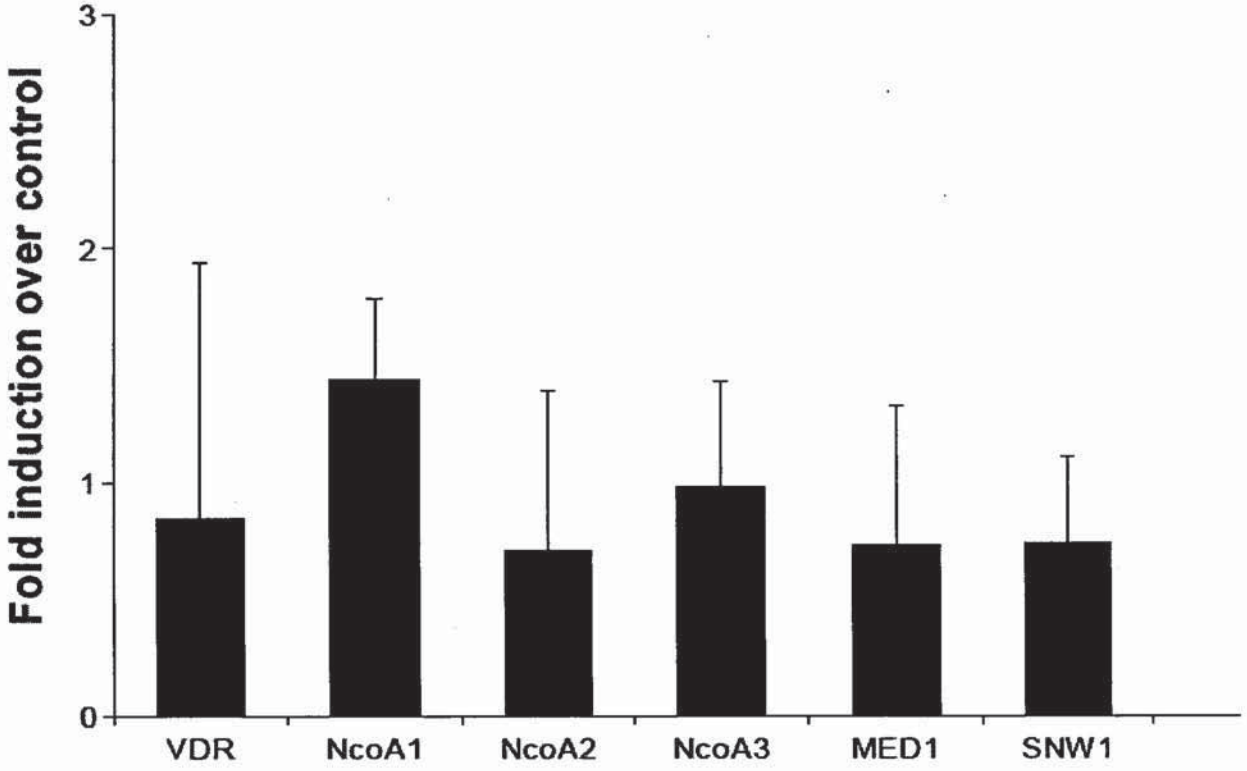


Figure 10

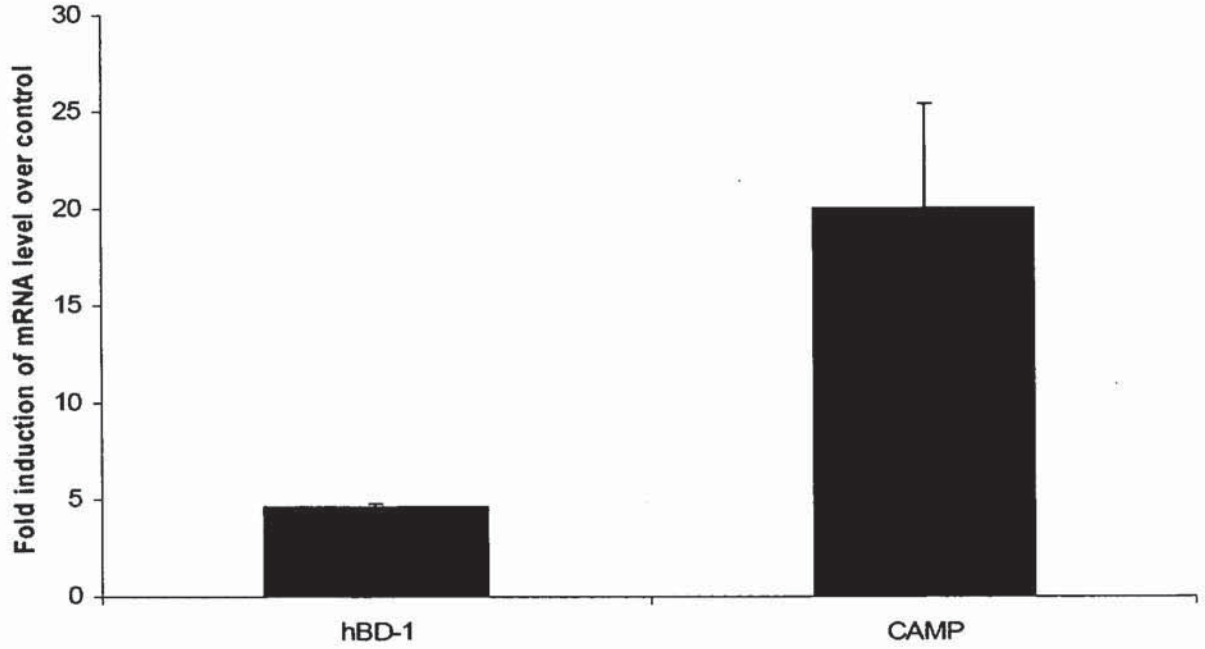
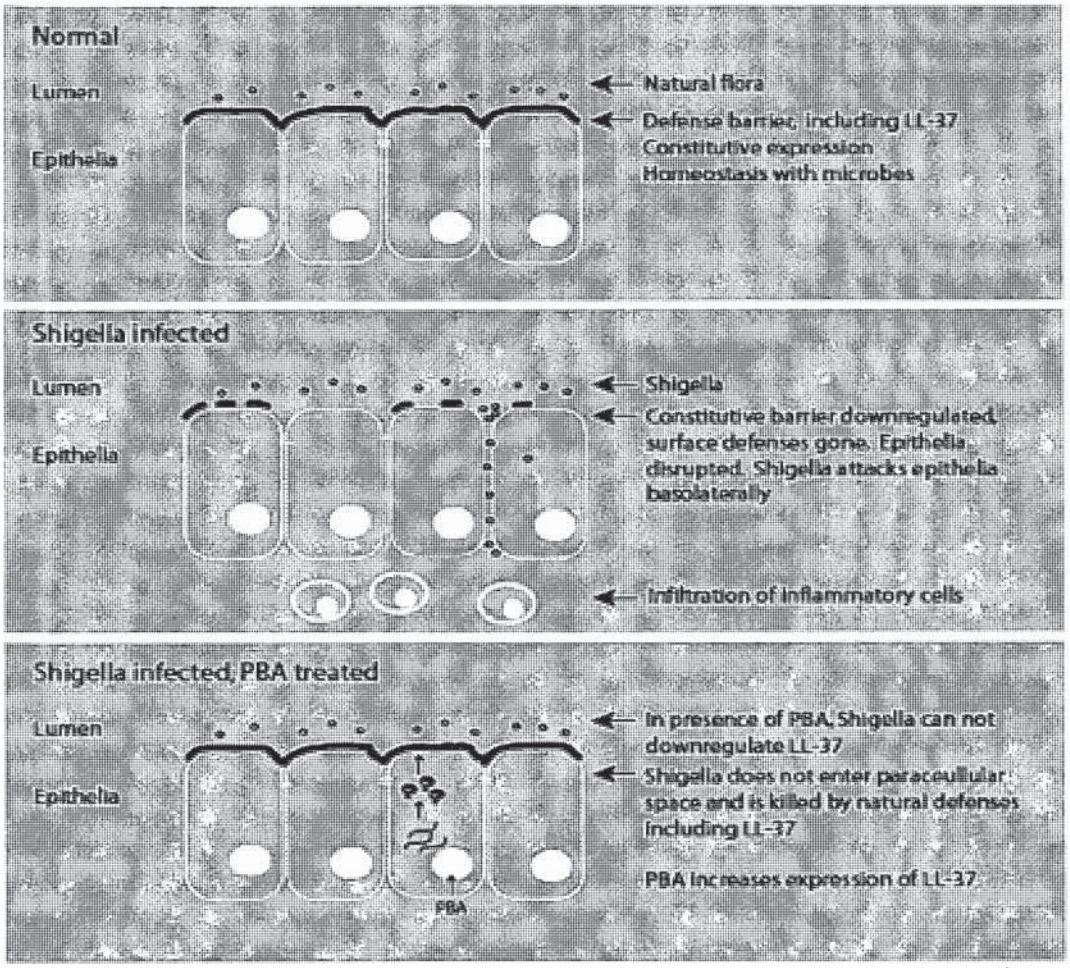


Figure 11

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
PBA
In presence of PBA, Shigella can not downregulate LL-37
Shigella does not enter paracellular space and is killed by natural defenses including LL-37
PBA increases expression of LL-37

ALTERNATIVE TO PTO/SB/08A/B
(Based on PTO 08-08 version)

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 1

Complete if Known	
Application Number	12/350,111
Filing Date	January 7, 2009
First Named Inventor	Bruce SCHARSCHMIDT
Art Unit	1614
Examiner Name	Not Yet Assigned
Attorney Docket Number	643982000100

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]
	1.	WO-2005/053607	06/2005			
	2.	WO-2009/087474	07/2009			

Examiner Signature		Date Considered	

*EXAMINER: Initial if information considered, whether or not citation 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 applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	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[2]
	3.	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	
	4.	COMTE et al., Journal of Mass Spectrometry (2002) 37(6):581-590	
	5.	LEE et al., Journal of Inherited Metabolic Disease (2008) 31(1):91	
	6.	Search and Examination Report for British Patent Application No. GB 0915545.8, dated 8 October 2009, 5 pages	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation 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 applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	5

Application Number	12/350,111
Filing Date	January 7, 2009
First Named Inventor	Bruce SCHARSCHMIDT
Art Unit	1614
Examiner Name	Not Yet Assigned
Attorney Docket Number	643982000100

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
- [ ] Fee Attached
- [ ] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [x] Information Disclosure Statement (Supplemental in 3 pages)
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

PTO Form SB/08a/b (1 page)

6 References

Remarks

CUSTOMER NO.: 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	February 2, 2010	Reg. No.	44,422

Patent
Docket No. 643982000100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Bruce SCHARSCHMIDT

Application No.: 12/350,111

Filing Date: January 7, 2009

For: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Examiner: Not Yet Assigned

Group Art Unit: 1614

Confirmation No.: 6290

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97 & § 1.98

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. § 1.97 and § 1.98, Applicant submits for consideration in the above-identified application the documents listed on the attached Form PTO/SB/08a/b. Copies of the documents are also submitted herewith. The Examiner is requested to make these documents of record.

The documents listed on the attached Form PTO/SB/08a/b were cited in a Search and Examination Report dated on October 8, 2009, directed to a counterpart international or foreign application and have not been previously cited.

This Information Disclosure Statement is submitted:

☐ With the application; accordingly, no fee or separate requirements are required.

☐ Before the mailing of a first Office Action after the filing of a Request for Continued Examination under 37 C.F.R. § 1.114. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.

☒ Within three months of the application filing date or before mailing of a first Office Action on the merits; accordingly, no fee or separate requirements are required. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.

☐ After receipt of a first Office Action on the merits but before mailing of a final Office Action or Notice of Allowance.

- ☐ A fee is required. Accordingly, a Fee Transmittal Form (PTO/SB/17) is attached to this submission.
- ☐ A Certification under 37 C.F.R. § 1.97(e) is provided above; accordingly; no fee is believed to be due.

☐ After mailing of a final Office Action or Notice of Allowance, but before payment of the Issue Fee.

- ☐ A Certification under 37 C.F.R. § 1.97(e) is provided above and a Fee Transmittal Form (PTO/SB/17) is attached to this submission.)

Applicants would appreciate the Examiner initialing and returning the Form PTO/SB/08a/b, indicating that the information has been considered and made of record herein.

The information contained in this Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98 is not to be construed as a representation that: (i) a complete 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 above information constitutes prior art to the subject invention.

In the unlikely event that the transmittal form is separated from this document and the Patent and Trademark Office determines that an extension and/or other relief (such as payment of a fee under 37 C.F.R. § 1.17 (p)) is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petition and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing 643982000100.

Dated: February 2, 2010

Respectfully submitted,

Electronic signature: /Michael G. Smith/
Michael G. Smith
Registration No.: 44,422
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 720-5113

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

Date of mailing *(day/month/year)*	30/12/2009
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	

DOCKETED: Resp to WO/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

RECEIVED
JAN 0 5 2010
MORRISON & FOERSTER
SAN DIEGO DOCKETING

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

INTERNATIONAL SEARCH REPORT

International 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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	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

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/210 278	Moreno de Vega, C Telephone No. +49 89 2399-7486

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

Electronic Acknowledgement Receipt

EFS ID:	7333982
Application Number:	12350111
International Application Number:	
Confirmation Number:	6290
Title of Invention:	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce SCHARSCHMIDT
Customer Number:	25225
Filer:	Michael Glenn Smith/Jessica Conen
Filer Authorized By:	Michael Glenn Smith
Attorney Docket Number:	643982000100
Receipt Date:	01-APR-2010
Filing Date:	07-JAN-2009
Time Stamp:	17:19:00
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	Miscellaneous Incoming Letter	643982000100TRANS.pdf	27882 339d9c0e45281455d2e6675fe4ac57648dc870fd	no	1

Warnings:	
Information:	

2	Transmittal Letter	643982000100SIDS.pdf	23948 94437b03489d6c59df89523af60dbd0d8baf641f	no	3
Warnings:					
Information:					
3	Information Disclosure Statement (IDS) Filed (SB/08)	643982000100SB08.pdf	30194 984ce1f7a90286c634b1c89dfe8c36a71807e6cb	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
4	NPL Documents	MACARTHUR2004MolecularGenMetab.pdf	561963 746a835e6027bdc8736af5b80d226dbb8662c7e6	no	7
Warnings:					
Information:					
5	NPL Documents	SIMMELL1986PediatricRsrch.pdf	557829 b0d328ee2f9ba774739c7ebfcf252554ed1161a2	no	5
Warnings:					
Information:					
6	NPL Documents	TANNER2007JInheritedMetabDispdf.pdf	633161 85f481690faad443082dda685381831aa8d3f44f	no	6
Warnings:					
Information:					
7	NPL Documents	PCT2009055256_ISRWO_12302009.pdf	730281 52fa5067d7e997849daab574b3fdb26cd8fa5757	no	13
Warnings:					
Information:					
		Total Files Size (in bytes):	2565258		

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.

ALTERNATIVE TO PTO/SB/08A/B
(Based on PTO 08-08 version)

Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				Application Number	12/350,111
				Filing Date	January 7, 2009
				First Named Inventor	Bruce SCHARSCHMIDT
				Art Unit	1614
(Use as many sheets as necessary)				Examiner Name	Not Yet Assigned
Sheet	1	of	1	Attorney Docket Number	643982000100

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature		Date Considered	

*EXAMINER: Initial if information considered, whether or not citation 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 applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	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[2]
	1.	MACARTHUR et al. (2004). Molecular Genetics and Metabolism 81(1):S67-S73	
	2.	SIMMELL et al. (1986). Pediatric Research 20(11):1117-1121	
	3.	TANNER et al. (2007). Journal of Inherited Metabolic Disease 30(5):716-721	
	4.	International Search Report and Written Opinion for PCT/US2009/055256, mailed 30 December 2009, 13 pages	

Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation 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 applicant.

[1]Applicant's unique citation designation number (optional). [2]Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	5

Application Number	12/350,111
Filing Date	January 7, 2009
First Named Inventor	Bruce SCHARSCHMIDT
Art Unit	1614
Examiner Name	Not Yet Assigned
Attorney Docket Number	643982000100

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
- [ ] Fee Attached
- [ ] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [x] Information Disclosure Statement (Supplemental in 3 pages)
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
- [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
- [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

PTO Form SB/08a/b (1 page)

4 References

Remarks

CUSTOMER NO.: 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	April 1, 2010	Reg. No.	44,422

Patent
Docket No. 643982000100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Bruce SCHARSCHMIDT

Application No.: 12/350,111

Filing Date: January 7, 2009

For: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Examiner: Not Yet Assigned

Group Art Unit: 1614

Confirmation No.: 6290

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97 & § 1.98

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Pursuant to 37 C.F.R. § 1.97 and § 1.98, Applicants submit for consideration in the above-identified application the documents listed on the attached Form PTO/SB/08a/b. Copies of the documents are also submitted herewith. The Examiner is requested to make these documents of record.

The documents listed on the attached Form PTO/SB/08a/b were cited in an International Search Report and Written Opinion mailed on December 30, 2009, directed to a counterpart international or foreign application and have not been previously cited.

This Information Disclosure Statement is submitted:

☐ With the application; accordingly, no fee or separate requirements are required.

☐ Before the mailing of a first Office Action after the filing of a Request for Continued Examination under 37 C.F.R. § 1.114. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.

☒ Within three months of the application filing date or before mailing of a first Office Action on the merits; accordingly, no fee or separate requirements are required. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.

☐ After receipt of a first Office Action on the merits but before mailing of a final Office Action or Notice of Allowance.

- ☐ A fee is required. Accordingly, a Fee Transmittal Form (PTO/SB/17) is attached to this submission.
- ☐ A Certification under 37 C.F.R. § 1.97(e) is provided above; accordingly; no fee is believed to be due.

☐ After mailing of a final Office Action or Notice of Allowance, but before payment of the Issue Fee.

- ☐ A Certification under 37 C.F.R. § 1.97(e) is provided above and a Fee Transmittal Form (PTO/SB/17) is attached to this submission.)

Applicants would appreciate the Examiner initialing and returning the Form PTO/SB/08a/b, indicating that the information has been considered and made of record herein.

The information contained in this Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98 is not to be construed as a representation that: (i) a complete 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 above information constitutes prior art to the subject invention.

In the unlikely event that the transmittal form is separated from this document and the Patent and Trademark Office determines that an extension and/or other relief (such as payment of a fee under 37 C.F.R. § 1.17 (p)) is required, Applicants petition for any required relief including

extensions of time and authorize the Commissioner to charge the cost of such petition and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing 643982000100.

Dated: April 1, 2010

Respectfully submitted,

Electronic signature: /Michael G. Smith/
Michael G. Smith
Registration No.: 44,422
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 720-5113

Application Data Sheet

Application Information

Application Type::	Regular
Subject Matter::	Utility
Suggested Group Art Unit::	Not Yet Assigned
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
Attorney Docket Number::	643982000100
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	15
Small Entity?::	Yes
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Bruce
Family Name::	SCHARSCHMIDT
City of Residence::	South San Francisco
State or Province of Residence::	CA
Country of Residence::	US
Street of mailing address::	601 Gateway Blvd. Ste. 200

City of mailing address::	South San Francisco
State or Province of mailing address::	CA
Postal or Zip Code of mailing address::	94080

Correspondence Information

Correspondence Customer Number::	25225

Representative Information

Representative Customer Number::	25225

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	An application claiming the benefit under 35 USC 119(e)	61/093,234	08/29/08

Foreign Priority Information

Assignee Information

Electronic Acknowledgement Receipt

EFS ID:	8481095
Application Number:	12350111
International Application Number:	
Confirmation Number:	6290
Title of Invention:	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce SCHARSCHMIDT
Customer Number:	25225
Filer:	Michael Glenn Smith/Jessica Conen
Filer Authorized By:	Michael Glenn Smith
Attorney Docket Number:	643982000100
Receipt Date:	23-SEP-2010
Filing Date:	07-JAN-2009
Time Stamp:	16:42:47
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	Application Data Sheet	643982000100ADSasfiled.pdf	34744 0646d469bd11394da367590c8b6a965aa38b9fcc	no	2

Warnings:	
Information:	

This is not an USPTO supplied ADS fillable form					
2	Oath or Declaration filed	643982000100SubDEC.pdf	217723 8d529ded5eefb58ea8947b7fceb16c05d6af a1bd	no	3
Warnings:					
Information:					
3	Miscellaneous Incoming Letter	643982000100MarkedFR.pdf	143039 97977d7ec980630c1f474aabb3cb24c9b89 cb2ce	no	3
Warnings:					
Information:					
4	Miscellaneous Incoming Letter	643982000100TRANS.pdf	28109 cb59b6602960eac0a5c4c99e3cc0de7b629 b0e32	no	1
Warnings:					
Information:					
5	Miscellaneous Incoming Letter	643982000100REQCFR.pdf	25763 417908f53867fb64939ee10ecb60fc078cb7 fae8	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	449378		

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

SUBSTITUTE DECLARATION FOR PATENT APPLICATION

As the below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

the specification of which was filed on January 7, 2009 as Application No. 12/350,111.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability as defined in 37 CFR 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

[x] no foreign applications have been filed

[ ] foreign application(s) have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			Yes No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

[ ] no U.S. provisional applications have been filed.

[x] U.S. provisional application(s) have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
61/093,234	August 29, 2008	x Yes No
		Yes No
		Yes No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of the United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information that is material to patentability as defined in 37 CFR 1.56 which became available to me between the filing date of the prior application and the national or PCT international filing date of this application:

[x] no U.S./PCT applications have been filed.

[ ] U.S./PCT application(s) have been filed as follows:

Application Number	Date of Filing	Status (Patented/Pending/Abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 25225

all of **Morrison & Foerster LLP**, 12531 High Bluff Drive, Suite 100, San Diego, California 92130-2040, jointly, and each of them severally, my attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith.

Please mail all correspondence to Michael G. Smith, whose address is:

Morrison & Foerster LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040

Please direct telephone calls to: Michael G. Smith at (858) 720-5113

Please direct facsimiles to: (858) 720-5125

Full name of sole or first inventor Bruce SCHARSCHMIDT	
Sole or first inventor's signature Bruce Scharschmidt	Date Sept 22 2010
Residence South San Francisco, California	
Citizenship US	
Mailing Address 601 Gateway Blvd. Ste. 200 South San Francisco, California 94080	

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
12/350,111	01/07/2009	1614	1686	643982000100	29	12

CONFIRMATION NO. 6290

25225
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

FILING RECEIPT

OC000000034140463

Date Mailed: 01/27/2009

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**

Applicant(s)

Bruce SCHARSCHMIDT, South San Francisco, CA;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/093,234 08/29/2008
and claims benefit of 61/048,830 04/29/2008

Foreign Applications

If Required, Foreign Filing License Granted: 01/21/2009

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 12/350,111**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

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

Title

METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Preliminary Class

514

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).

Docket No.: 643982000100
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Bruce SCHARSCHMIDT

Application No.: 12/350,111 Confirmation No.: 6290

Filed: January 7, 2009 Art Unit: 1614

For: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS Examiner: Not Yet Assigned

REQUEST FOR CORRECTED FILING RECEIPT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicant hereby requests that a corrected Filing Receipt be issued in the above-identified patent application. The official Filing Receipt received by Applicant, a copy of which is attached hereto, has an error in the priority information.

Under the Domestic Priority data as claimed by applicant please delete "and claims benefit of 61/048,830"

A copy of the Filing Receipt received by the Applicant with the requested changes noted in red-ink, a Substitute Declaration and a copy of the Application Data Sheet as filed on January 7, 2009 is enclosed. Please refer to the Application Data Sheet for verification of the correct data.

Applicant additionally requests that all pertinent U.S. Patent and Trademark Office records relating to the subject application be changed to reflect this correction.

In the unlikely event that the transmittal letter is separated from this request and the Patent Office determines that a fee is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing Attorney Docket No. 643982000100.

Dated: September 23, 2010

Respectfully submitted,

Electronic signature: /Michael G. Smith/
Michael G. Smith
Registration No.: 44,422
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, California 92130-2040
(858) 720-5113

PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	11

Application Number	12/350,111
Filing Date	January 7, 2009
First Named Inventor	Bruce SCHARSCHMIDT
Art Unit	1614
Examiner Name	Not Yet Assigned
Attorney Docket Number	643982000100

ENCLOSURES (*Check all that apply*)

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☒ Other Enclosure(s) (please Identify below):

Request for Corrected Filing Receipt (2 pages)
Copy of Marked Filing Receipt (3 pages)
Copy of Originally filed Application Data Sheet (2 pages)
Substitute Declaration (3 pages)

Remarks

CUSTOMER NO.: 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	September 23, 2010	Reg. No.	44,422

PTO/SB/96 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Bruce Scharschmidt

Application No./Patent No.: 12/350,111 Filed/Issue Date: January 7, 2009

Titled: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

UCYCLYD PHARMA, INC. , a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest in;

2. [ ] an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or

3. [ ] an assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent application/patent identified above by virtue of either:

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.

OR

B. [X] 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 To: Hyperion Therapeutics
The document was recorded in the United States Patent and Trademark Office at
Reel 022305 , Frame 0387 , or for which a copy thereof is attached.

2. From: Hyperion Therapeutics To: UCYCLYD PHARMA, INC.
The document was recorded in the United States Patent and Trademark Office at
Reel 025031 , Frame 0014 , or for which a copy thereof is attached.

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[ ] Additional documents in the chain of title are listed on a supplemental sheet(s).

[X] As required by 37 CFR 3.73(b)(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.

/Michael G. Smith/	October 27, 2010
Signature	Date
Michael G. Smith	Attorney of Record
Printed or Typed Name	Title

Electronic Acknowledgement Receipt

EFS ID:	8712643
Application Number:	12350111
International Application Number:	
Confirmation Number:	6290
Title of Invention:	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce SCHARSCHMIDT
Customer Number:	25225
Filer:	Michael Glenn Smith/Jessica Conen
Filer Authorized By:	Michael Glenn Smith
Attorney Docket Number:	643982000100
Receipt Date:	27-OCT-2010
Filing Date:	07-JAN-2009
Time Stamp:	19:08:31
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	Miscellaneous Incoming Letter	643982000100TRANS.pdf	27819 6790ccf8ed7676714192cd388e005cf4125c9998	no	1

Warnings:	
Information:	

2	Power of Attorney	643982000100POA.pdf	54759 636d1491188f01d1724b79b6d00776b2a90351b	no	1
Warnings:					
Information:					
3	Assignee showing of ownership per 37 CFR 3.73(b).	643982000100373b.pdf	22234 fda382ed21d66abba9d1cbb289e61d6217436d28	no	1
Warnings:					
Information:					
		Total Files Size (in bytes):	104812		

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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.

PTO/SB/80 (01-06)
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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(b).

I hereby appoint:

[x] Practitioners associated with the Customer Number: 25225

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 assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

[X] The address associated with Customer Number: 25225

OR

[ ] Firm or Individual Name					
Address					
City		State		Zip	
Country		Telephone		Email	

Assignee Name and Address:
UCYCLYD PHARMA, INC.
7720 North Dobson Road
Scottsdale, AZ 85256

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, 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	Len Smith	Date	10/12-010
Name	Len Smith	Telephone	480-291-5892
Title	Principal Intellectual Property Counsel		

Attorney Docket No.: 643983000100

sd-532405

PTO/SB/21 (07-09)
Approved for use through 07/31/2012. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	3

Application Number	12/350,111
Filing Date	January 7, 2009
First Named Inventor	Bruce SCHARSCHMIDT
Art Unit	1614
Examiner Name	Not Yet Assigned
Attorney Docket Number	643982000100

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [x] Power of Attorney, Revocation Change of Correspondence Address (1 pages)
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

  PTO form 3.73(b) (1 page)

Remarks

CUSTOMER NO.: 25225

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	October 27, 2010	Reg. No.	44,422

REMARKS

In the amendment herein, claims 1, 3-4, 6-8, 10, and 11 are amended. Claims 5, 9 and 12-29 have been cancelled. New claims 30-44 have been added. Accordingly, claims 1-4, 6-8, 10-11, and 30-44 are pending. No new matter has been introduced. Support for the amendments to the claims and the new claims is found in the claims as originally filed, and throughout the specification including pages 10-14. No new matter has been introduced.

Examination of the claims as amended herein is respectfully requested.

If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 643982000100. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: May 12, 2011

Respectfully submitted,

E-Signature: /Madeline I. Johnston/
Madeline I. Johnston
Registration No.: 36,174
MORRISON & FOERSTER LLP
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pa-1459526

Supplemental Application Data Sheet

Application Information

Application number::	12/350,111
Filing Date::	01/07/09
Application Type::	Regular
Subject Matter::	Utility
Suggested Group Art Unit::	~~Not Yet Assigned~~1651
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
Attorney Docket Number::	643982000100
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	15
Small Entity?::	~~Yes~~No
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Bruce
Family Name::	SCHARSCHMIDT
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State or Province of Residence::	CA

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Street of mailing address::	601 Gateway Blvd. Ste. 200
City of mailing address::	South San Francisco
State or Province of mailing address::	CA
Postal or Zip Code of mailing address::	94080

Correspondence Information

Correspondence Customer Number::	25225

Representative Information

Representative Customer Number::	25225

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	An application claiming the benefit under 35 USC 119(e)	61/093,234	08/29/08

Foreign Priority Information

Assignee Information

Assignee name::	UCYCLYD PHARMA, INC.
Street of mailing address::	7720 North Dobson Road
City of mailing address::	Scottsdale
State or Province of mailing address::	AZ
Country of mailing address::	US
Postal or Zip Code of mailing address::	85256

Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.			
Signature	/Madeline I. Johnston/	Date	May 12, 2011
Name (Print/Type)	Madeline I. Johnston	Registration No. (Attorney/Agent)	36,174

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings of claims in the application:

Claim 1 (Currently Amended): A method to determine an effective dosage of a phenylacetic acid (PAA) prodrug selected from glyceryl tri-[4-phenylbutyrate] (HPN-100) and phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof for a patient in need of treatment for a nitrogen retention disorder selected from urea cycle disorder and hepatic encephalopathy, which comprises monitoring the effect of ~~an initial~~ a dosage of ~~HPN-100~~ the prodrug in a patient to whom the prodrug has been administered,

wherein monitoring the effect ~~consists essentially of~~ comprises determining the patient's urinary phenylacetyl glutamine (PAGN) output;

and determining from the urinary PAGN output ~~whether and/or how to adjust~~ the ~~initial~~ effective dosage of ~~HPN-100~~ the prodrug to produce a desired ammonia scavenging effect.

Claim 2 (Original): The method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine.

Claim 3 (Currently Amended): The method of claim 1, wherein the ~~nitrogen retention disorder is chronic hepatic encephalopathy or a urea cycle disorder~~ method comprises calculating the dosage of prodrug based on a utilization efficiency for prodrug conversion into PAGN of about 60% to about 75%.

Claim 4 (Currently Amended): The method of claim 1, wherein the prodrug is HPN-100 , and wherein administering the effective dosage of HPN-100 to the patient produces a normal plasma ammonia level in the patient.

Claim 5 (Cancelled).

Claim 6 (Currently Amended): A method to determine a dosage of a phenylacetic acid (PAA) prodrug selected from glyceryl tri-[4-phenylbutyrate] (HPN-100) and phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof for a patient having a nitrogen retention disorder selected from urea cycle disorder and hepatic encephalopathy, which comprises measuring urinary excretion of phenylacetyl glutamine (PAGN) in a patient to whom the PAA prodrug has been administered and calculating the dosage of ~~HPN-100~~ the PAA prodrug based on a utilization efficiency for ~~HPN-100~~ the prodrug conversion into PAGN of about 60% to about 75%.

Claim 7 (Currently Amended): The method of claim 6, wherein the dosage of ~~HPN-100~~ the PAA prodrug is calculated from the patient's dietary protein intake.

Claim 8 (Currently Amended): The method of claim 7, wherein the dosage of ~~HPN-100~~ the PAA prodrug is ~~reduced~~ adjusted to account for the patient's residual urea synthesis capacity.

Claim 9 (Cancelled).

Claim 10 (Currently Amended): The method of claim [9] 1, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof.

Claim 11 (Currently Amended): The method of claim [9] 1, wherein the PAA prodrug is HPN-100.

Claims 12-29 (Cancelled).

Claim 30 (New): The method of claim 1, wherein the PAA prodrug is sodium phenylbutyrate.

Claim 31 (New): The method of claim 1, wherein the nitrogen retention disorder is urea cycle disorder.

Claim 32 (New): The method of claim 1, wherein the nitrogen retention disorder is hepatic encephalopathy.

Claim 33 (New): The method of claim 6, wherein the nitrogen retention disorder is urea cycle disorder.

Claim 34 (New): The method of claim 6, wherein the nitrogen retention disorder is hepatic encephalopathy.

Claim 35 (New): The method of claim 6, wherein the prodrug is HPN-100.

Claim 36 (New): The method of claim 6, wherein the prodrug is PBA or a pharmaceutically acceptable salt thereof.

Claim 37 (New): The method of claim 6, wherein the prodrug is sodium phenylbutyrate.

Claim 38 (New): A method of administering a phenylacetic acid (PAA) prodrug selected from glyceryl tri-[4-phenylbutyrate] (HPN-100) and phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof to a patient having a nitrogen retention disorder selected from urea cycle disorder and hepatic encephalopathy, the method comprising determining urinary phenylacetylglutamine (PAGN) excretion of the patient following administration of the PAA prodrug, determining a dose of the PAA prodrug based on the PAGN excretion, and administering the dose to the patient.

Claim 39 (New): The method of claim 38, wherein the dosage of the PAA prodrug is based on a utilization efficiency for the PAA prodrug conversion into PAGN of about 60% to about 75%.

Claim 40 (New): The method of claim 38, wherein PBA or a pharmaceutically acceptable salt thereof is administered.

Claim 41 (New): The method of claim 38, wherein sodium phenylbutyrate is administered.

Claim 42 (New): The method of claim 38, wherein HPN-100 is administered.

Claim 43 (New): The method of claim 38, wherein the disorder is urea cycle disorder.

Claim 44 (New): The method of claim 38, wherein the disorder is hepatic encephalopathy.

Electronic Acknowledgement Receipt

EFS ID:	10075819
Application Number:	12350111
International Application Number:	
Confirmation Number:	6290
Title of Invention:	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
First Named Inventor/Applicant Name:	Bruce SCHARSCHMIDT
Customer Number:	25225
Filer:	Madeline I. Johnston/Farah O'Sullivan
Filer Authorized By:	Madeline I. Johnston
Attorney Docket Number:	643982000100
Receipt Date:	12-MAY-2011
Filing Date:	07-JAN-2009
Time Stamp:	20:53:08
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	Miscellaneous Incoming Letter	64398-20001_00__Notification_of_Loss_of_Entitlement_to_Small_Entity_Status_and_Payme.pdf	21318 86133a37642df4980c796b5c82e8865f1e4b2182	no	3

Warnings:	
Information:	

2	Petition to make special based on Age/ Health	64398-20001_00_Petition_to_Make_Special-Applicants_Age.pdf	42800 35427391bec65ed6e668d9ce641b392e0a41454c	no	3
Warnings:					
Information:					
3	Miscellaneous Incoming Letter	64398-20001_00__Fee_Transmittal.pdf	44324 fa3a97a92c047363f956534dbc48b1c15f8acb03	no	1
Warnings:					
Information:					
4	Transmittal Letter	64398-20001_00_SIDS_Cover_Letter.pdf	20261 c6ce4df6c8a735c2f62d39eb11f3e246e199e334	no	3
Warnings:					
Information:					
5	Information Disclosure Statement (IDS) Filed (SB/08)	64398-20001_00_SIDS_SB08_--Final_Draft.pdf	67971 4a881f81719c62ea7fe8d63fd325dec68beec4f7	no	4
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
6	Foreign Reference	ForeignRefs_2_3.pdf	10398228 062535a4c5211f42bb70bccb1c6a8fd8b3143f90	no	176
Warnings:					
Information:					
7	NPL Documents	NonPatent_4_15__.pdf	21846114 6531f66b83a865182413719e93d905b621537a0e	no	178
Warnings:					
Information:					
8	NPL Documents	NonPatent_16_30.pdf	7270792 b1aeb2cc5de3d350a107a152657738809d12554d	no	87
Warnings:					
Information:					
9	NPL Documents	NonPatent_31_60__.pdf	9759028 a5dca5cdf95f89c92a9e1431ec05327e6ae792f0	no	121
Warnings:					
Information:					
10	Miscellaneous Incoming Letter	64398-20001_00__Transmittal.pdf	25712 3250cdecc238bc1c779609cb37c44fa878ea0a90	no	1

Warnings:					
Information:					
11	Application Data Sheet	64398-20001_00__SADS.pdf	15926 f6c7528dd37c6189521e08427dc677b1c32d85c7	no	3
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
12		64398-20001_00_1st_Preliminary_Amendment.pdf	26802 e1fae41bbc31267937597134aae0f13f73de16d1	yes	6

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	5
Applicant Arguments/Remarks Made in an Amendment	6	6

Warnings:	
Information:	
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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.

PTO/SB/06 (07-06)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 12/350,111	Filing Date 01/07/2009	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☒		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			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 $250 ($125 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			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	05/12/2011	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)	OR	OTHER THAN SMALL ENTITY RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 24	Minus	** 29	= 0	X $26 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 3	Minus	***12	= 0	X $110 =	0	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	0	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		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 number found in the appropriate box in column 1.

Legal Instrument Examiner:
/LINDA WISE/

This collection of information is required by 37 CFR 1.16. 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, 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.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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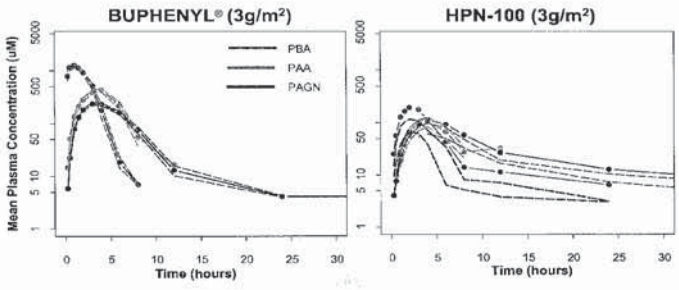
(54) **Title**: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

FIGURE 4



In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving BUPHENYL® (sodium phenylbutyrate) (sodium PBA) at 3g/m² dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100. In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately12 hours, and fall generally close to the PAGN curves up to that time.

(57) **Abstract**: The invention provides a method for determining a dose and schedule and making dose adjustments of PBA prodrugs used to treat nitrogen retention states, or ammonia accumulation disorders, by measuring urinary excretion of phenylacetylglutamine and/or total urinary nitrogen. The invention provides methods to select an appropriate dosage of a PBA prodrug based on the patient's dietary protein intake, or based on previous treatments administered to the patient. The methods are applicable to selecting or modifying a dosing regimen for a subject receiving an orally administered ammonia scavenging drug.

METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Cross-Reference to Related Applications

[0001] This application claims benefit of priority to U.S. Provisional application serial number 61/093,234, filed August 29, 2008, which is incorporated herein by reference in its entirety. This application is also related to the U.S. provisional patent application entitled "Treating special populations having liver disease with nitrogen-scavenging compounds," naming Sharron Gargosky as inventor, serial number 61/048,830, filed on April 29, 2008.

Technical Field

[0002] This invention relates to treatment of patients with nitrogen retention states, in particular urea cycle disorders (UCDs) and cirrhosis complicated by hepatic encephalopathy (HE), using administered compounds that assist in elimination of waste nitrogen from the body. The compounds can be orally administered small-molecule drugs, and the invention provides methods for delivering these compounds and selecting suitable dosages for a patient.

Background Art

[0003] Drug dosing is usually based upon measurement of blood levels of the active drug species in conjunction with clinical assessment of treatment response. However, the present invention is based on evidence that for certain prodrugs of phenylacetic acid (PAA), measuring the blood level of the prodrug (e.g. PBA) or of PAA formed from it is unreliable. In addition, assessment of treatment effect by measuring levels of ammonia in the blood is inconvenient, because it requires withdrawing multiple blood samples under carefully controlled conditions. Because blood ammonia levels are affected by various factors including dietary protein, they also fail to provide a direct measure of how much ammonia the drug is mobilizing for elimination. The invention demonstrates that prodrugs of phenylbutyric acid (PBA) behave similarly to sodium PBA, in that measuring PBA levels is unreliable for assessing their effectiveness. This invention provides a novel method for dosing in patients with nitrogen retention states, in particular patients with liver disease and clinical manifestations of hepatic encephalopathy and patients with UCDs. It is particularly applicable to prodrugs that liberate or are metabolized to form phenylacetic acid, i.e., prodrugs of PAA, and those prodrugs that are metabolized to form PBA.

[0004] Hepatic encephalopathy refers to a spectrum of neurologic signs and symptoms which frequently occur in patients with cirrhosis or certain other types of liver disease.

[0005] Urea cycle disorders comprise several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. The enzymes including their Enzyme Commission (EC) numbers and modes of inheritance include the following:

- Carbamyl phosphate synthetase (CPS; EC Number 6.3.4.16; autosomal recessive),
- ornithine transcarbamylase (OTC; EC Number 2.1.3.3; X-linked),
- argininosuccinate synthetase (ASS; EC Number 6.3.4.5; autosomal recessive),
- argininosuccinate lyase (ASL; EC Number 4.3.2.1; autosomal recessive),
- arginase (ARG; EC Number 3.5.3.1; autosomal recessive), and
- N-acetyl glutamine synthetase (NAGS 1; EC Number 2.3.1.1; autosomal recessive)

[0006] Mitochondrial transporter deficiency states which mimic many features of urea cycle enzyme deficiencies include the following:

- Ornithine translocase deficiency (hyperornithinemia, hyperammonemia, homocitrullinuria or HHH Syndrome)
- Citrin (aspartate glutamate transporter) deficiency

[0007] The common feature of UCD and hepatic encephalopathy that render them treatable by methods of the invention is an accumulation of excess waste nitrogen in the body, and hyperammonemia. In normal individuals, the body's intrinsic capacity for waste nitrogen excretion is greater than the body's waste nitrogen production, so waste nitrogen does not accumulate and ammonia does not build up to harmful levels. For patients with nitrogen retention states such as UCD or HE, the body's intrinsic capacity for waste nitrogen excretion is less than the body's waste nitrogen production based on a normal diet that contains significant amounts of protein. As a result, nitrogen builds up in the body of a patient having a nitrogen retention disorder, and usually results in excess ammonia in the blood. This has various toxic effects; drugs that help eliminate the excess ammonia are an important part of an overall management strategy for such disorders.

[0008] To avoid build-up of ammonia to toxic levels in patients with nitrogen retention states, dietary intake of protein (a primary source of exogenous waste nitrogen) must be balanced by the patient's ability to eliminate excess ammonia. Dietary protein can be limited, but a healthy diet requires a significant amount of protein, particularly for growing

children; thus in addition to controlling dietary protein intake, drugs that assist with elimination of nitrogen are used to reduce ammonia build-up (hyperammonemia). The capacity to eliminate excess ammonia in treated patients can be considered the sum of the patient's endogenous capacity for nitrogen elimination (if any) plus the amount of additional nitrogen-elimination capacity that is provided by a nitrogen scavenging drug. The methods of the invention use a variety of different drugs that reduce excess waste nitrogen and ammonia by converting it to readily-excreted forms, such as phenylacetyl glutamine (PAGN). In some embodiments, the invention relates to methods for determining or adjusting a dosage of an oral drug that forms PAA *in vivo*, which is converted into PAGN, which is then excreted in urine and thus helps eliminate excess nitrogen.

[0009] Based on prior studies in individual UCD patients (e.g. Brusilow, Pediatric Research, vol. 29, 147-50 (1991); Brusilow and Finkelstien, J. Metabolism, vol. 42, 1336-39 (1993)) in which 80-90% of the nitrogen scavenger sodium phenylbutyrate was reportedly excreted in the urine as PAGN, current treatment guidelines typically either assume complete conversion of sodium phenylbutyrate or other PAA prodrugs to PAGN (e.g. Berry et al., J. Pediatrics, vol. 138, S56-S61 (2001)) or do not comment on the implications of incomplete conversion for dosing (e.g. Singh, Urea Cycle Disorders Conference Group '*Consensus Statement from a Conference for the Management of Patients with Urea Cycle Disorders*', Suppl to J Pediatrics, vol. 138(1), S1-S5 (2001)).

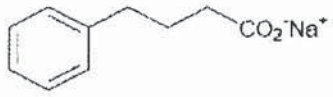
[0010] Current treatment guidelines recommend 4 times per day dosing, based on the fact that PBA is absorbed rapidly from the intestine when administered in the form of sodium PBA and exhibits a short half life in the bloodstream (Urea Cycle Disorders Conference Group 'Consensus Statement' 2001)

[0011] Current recommendations for sodium phenylbutyrate dosing indicate that dosage should not exceed 600 mg/kg (for patients weighing up to 20 kg) or in any case 20 grams total.

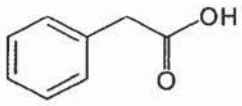
Disclosure of Embodiments of the Invention

[0012] The invention provides a novel approach for determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs, including sodium phenylbutyrate and glyceryl tri-[4-phenylbutyrate] (HPN-100), based upon the urinary excretion of the drug metabolite phenylacetylglutamine (PAGN) and/or total urinary nitrogen. It is based in part on the discoveries that bioavailability of these drugs as conventionally assessed based on systemic blood levels of the drugs themselves or of the active species produced *in vivo* from these drugs does not accurately predict removal of

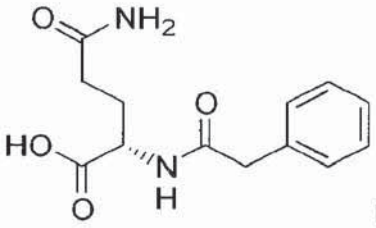
waste nitrogen or reduction of plasma ammonia in healthy human volunteers, adults with liver disease, or patients with UCDs receiving ammonia scavenging drugs as defined below and that conversion of orally administered sodium phenylbutyrate (NaPBA, or sodium PBA) to PAGN to urinary PAGN is incomplete, typically about 60-75%. Prodrugs of phenylbutyrate (PBA, the active ingredient in BUPHENYL®(sodium phenylbutyrate), which is the sodium salt of PBA along with small amounts of inert ingredients), which is itself a prodrug of phenylacetic acid (PAA), are especially subject to the effects described herein.



phenylbutyrate



Phenylacetic acid



Phenylacetylglutamine

[0013] As used herein "ammonia scavenging drugs" is defined to include all orally administered drugs in the class which contain or are metabolized to phenylacetate. Thus, the term includes at least phenylbutyrate, BUPHENYL® (sodium phenylbutyrate), AMMONAPS®, butyroyloxymethyl-4-phenylbutyrate, glyceryl tri-[4-phenylbutyrate] (HPN-100), esters, ethers, and acceptable salts, acids and derivatives thereof. These drugs reduce high levels of endogenous ammonia by providing phenylacetic acid in vivo, which is metabolized efficiently to form phenylacetyl glutamine (PAGN). PAGN is efficiently excreted in urine, carrying away two equivalents of nitrogen per mole of PAA converted to PAGN. References herein to sodium phenylbutyrate are understood to include reference to the drug product BUPHENYL®, and BUPHENYL® was used for the Examples herein wherever test subjects were treated with sodium phenylbutyrate. Thus the sodium PBA dosages used in the Examples generally refer to a dosage of BUPHENYL®, and the amounts of sodium phenylbutyrate in those Examples should be interpreted accordingly. Note that the terms 'ammonia scavenger' and 'nitrogen scavenger' are used interchangeably in this

invention, reflecting the fact that the drugs described herein lower blood ammonia through elimination of waste nitrogen in the form of PAGN.

[0014] In some embodiments, the invention uses prodrugs that can be converted into PAA within the body. Sodium phenylbutyrate (sodium PBA) is one such drug; it is converted by oxidative mechanisms into PAA in the body. HPN-100 is another such drug: it can be hydrolyzed to release PBA, which in turn can be oxidized to form PAA. Thus, HPN-100 is a prodrug of PBA, and also a prodrug of PAA. Clinical evidence demonstrates that HPN-100 is converted into PAA in the body as expected, and that PAA is then linked to a molecule of glutamine and converted into PAGN, which is eliminated in the urine as predicted. This process can be summarized as follows:

HPN-100 → 3 PBA → 3 PAA

PAA + glutamine → PAGN.

[0015] PAGN is mainly excreted in the subject's urine, and removes two molecules of ammonia per molecule of excreted PAGN. Each HPN-100 molecule forms three PAA molecules, so each molecule of HPN-100 can promote excretion of six molecules of ammonia. The clinical results suggest that conversion of HPN-100 into PBA and PAA is efficient and fairly rapid, but surprisingly suggest that some conversion of HPN to PAGN may occur before the HPN-100 (or PBA, or PAA derived from PBA) enters systemic circulation. As a result, systemic levels of PAA or PBA are not reliably correlated with the efficacy of HPN-100 as an ammonia scavenger.

[0016] In some embodiments, the invention uses a prodrug of PBA, including HPN-100 and other esters of phenylbutyrate. The PBA prodrug is thus a prodrug of a prodrug, since PBA acts to scavenge ammonia after it is converted to PAA and is thus considered a prodrug of PAA. In some embodiments, the PBA prodrug is an ester of phenylbutyrate, such as those described below; a preferred PBA prodrug for use in the invention is HPN-100. These compounds can be made and used by methods disclosed in U.S. Patent No. 5,968,979, which is incorporated herein by reference for its description of these compounds and methods for their administration.

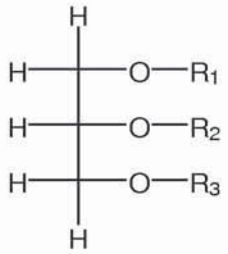
[0017] Where an 'equal molar' or 'equimolar' amount of a second drug is to be used along with or instead of a certain amount of a first drug, the amount of each drug is calculated on a molar basis, and the equimolar amount of the second drug is the amount that produces an equal molar amount of active drug *in vivo*. Where one of the drugs is a prodrug, the amount of prodrug will typically refer to the molar amount of the active species

formed from that prodrug. That active species is usually PAA for the prodrugs described herein, and the molar amount of a prodrug corresponds to the amount of PAA that would form in the body from that amount of the prodrug, assuming complete conversion into PAA occurs *in vivo*. Thus, for example, a molecule of HPN-100 can be metabolized by ester hydrolysis followed by oxidation to form three molecules of PAA, so a mole of HPN-100 would be considered equimolar to three moles of PAA. Similarly, since HPN-100 hydrolyzes to form three molecules of PBA (and one molecule of glycerin), an equimolar amount of HPN-100 would be one-third of the molar amount of PBA.

[0018] The following Table sets forth amounts of HPN-100 that correspond to equimolar amounts of certain relevant doses of BUPHENYL® (sodium phenylbutyrate). Note that the conversion of the dose of sodium PBA to the dose of HPN-100 involves correction for their different chemical forms [i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium; (sodium PBA [g] x 0.95 = HPN-100 [g])] as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

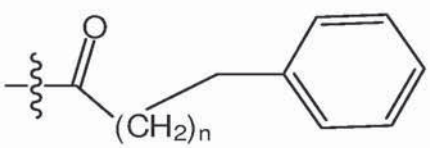
BUPHENYL® (sodium PBA)	**HPN-100 PBA Equivalent Dose (mg)**	**HPN-100 PBA Equivalent Dose (mL)**
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[0019] The present invention can use prodrugs of the formula (I):

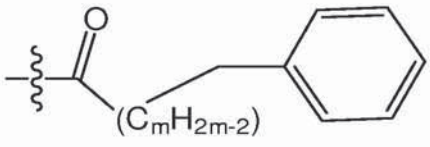


(I)

wherein R_1, R_2, and R_3 are independently, H,

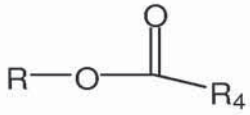


or



and n is zero or an even number, m is an even number and at least one of R_1, R_2, and R_3 is not H. For each R_1, R_2, or R_3, n or m is independently selected, so the R_1, R_2, and R_3 groups in a compound of formula I do not have to be identical. The preferred compounds are those wherein none of R_1, R_2, and R_3 is H, and frequently each n or m for a particular embodiment is the same, i.e., R_1, R_2, and R_3 are all the same. The advantage over the prior art of decreased dosage is greater with such triesters, and having all three acyl groups the same reduces issues related to mixtures of isomers. Moreover, the triol backbone liberated by hydrolysis of the esters is glycerol, a normal constituent of dietary triglyceride which is non-toxic.

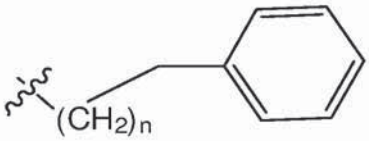
[0020] The present invention also utilizes phenylbutyrate and phenylacetate prodrugs of the formula II:



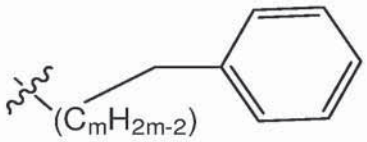
(II)

wherein R is a C_1-C_{10} alkyl group,

R_4 is



or

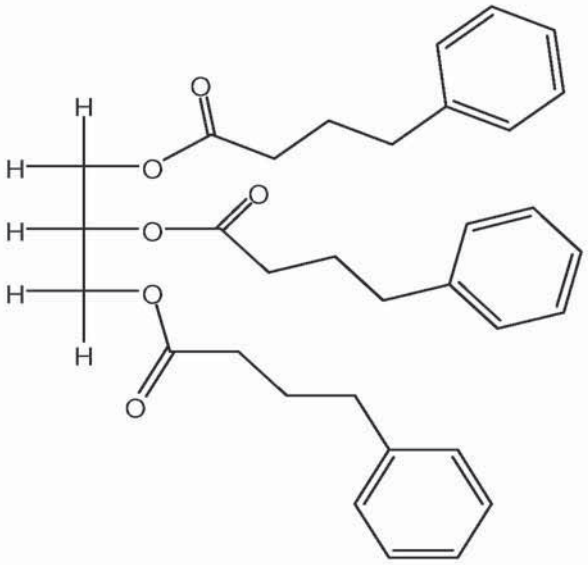


and n is zero or an even number, and m is an even number.

[0021] In Formula II, R can be, for example, ethyl, propyl, isopropyl, n-butyl, and the like.

[0022] The compounds of the invention are esters of the congeners of phenylalkanoic and phenylalkenoic acids having an even number of carbon atoms in the alkanoic acid portion, which include phenylacetic acid esters and those of phenylbutyric acid, etc., which can be converted by efficient beta-oxidation processes to phenylacetic acid in the body. They are thus prodrugs for phenylacetic acid. Where n is 2 or 4, the esters are also prodrugs for phenylbutyric acid. Preferably the alkylene or alkenylene carboxylate group contains 24 or fewer carbon atoms, so n or m is less than 24. In some embodiments, n and m are 0, 2, 4 or 6, and in some preferred embodiments n or m is 2.

[0023] Certain preferred embodiments of the invention use HPN-100 (Formula III):



(III)

[0024] Total daily dosage of prodrugs like sodium PBA can often be selected according to the amount needed to provide an appropriate amount of the active species, if that amount is known or can be determined. PBA is a prodrug for PAA; therefore, an initial dose of PBA could be selected if an effective dosage of PAA were known, taking into account the fraction of PBA that is converted into PAA and ultimately into PAGN. If a subject has been treated with PAA or a prodrug that forms PAA in the body, the amount of the previously used drug that was effective provides a possible starting point for selecting a dosage of a new prodrug of PAA. In this same patient, after the new prodrug is administered at the expected PAA dose equivalence, the PAA levels in the subject could be monitored and the dose of the prodrug adjusted until the same plasma level of PAA that was effective with the previous treatment is achieved. However, the current invention is based in part on finding that plasma PAA and PBA levels are not well correlated with the dose of a PBA prodrug administered or with ammonia elimination; for monitoring a dosing level of a PBA prodrug, one should not rely upon these parameters to assess the effectiveness of the prodrug. While not bound by the underlying theory, explanations for this effect (i.e. the inconsistent relationship between ammonia scavenging and PBA and/or PAA blood levels) are provided herein.

[0025] The following Table provides data from three clinical test groups showing the inconsistent relationship between plasma PAA and PBA levels among healthy volunteers, patients with cirrhosis and UCD patients, despite that fact that, as described in detail below,

all groups exhibited similar ammonia scavenging activity based on urinary excretion of PAGN. Overall, this shows that urinary PAGN provides a convenient method for monitoring ammonia elimination induced by the administered drug, which does not require drawing blood and directly relates to the actual nitrogen elimination provided by the administered nitrogen scavenging drug without being influenced by the many other factors that can affect plasma ammonia levels.

Plasma Pharmacokinetics of PBA, PAA, and PAGN Comparison across Studies

Analyte	Treatment	C_{max} (µg/mL)	T_{max} (h)	T½ (h)	AUC_{24} (µg·h/mL)
Healthy Volunteers (Single Dose – 3 g/m²/day PBA Mole Equivalent)					
PBA	Sodium PBA	221.0	0.9	0.7	542.6
	HPN-100	37.0	2.4	1.9	137.2
PAA	Sodium PBA	58.8	3.9	1.2	279.8
	HPN-100	14.9	4.0	NC	70.9
PAGN	Sodium PBA	63.1	3.2	1.7	395.1
	HPN-100	30.2	4.0	NC	262.1
Healthy Volunteers and Cirrhotic Patients (100 mg/kg BID) [1]					
PBA	Child-Pugh A	42.8	2.3	1.2	131.7
	Child-Pugh B	41.8	2.9	3.4	189.5
	Child-Pugh C	44.3	3.1	1.9	192.1
	Volunteers	29.8	3.0	2.1	132.7
PAA	Child-Pugh A	33.2	3.8	1.8	168.8
	Child-Pugh B	30.8	4.5	2.8	252.4
	Child-Pugh C	53.1	4.8	7.7	579.9
	Volunteers	25.5	3.6	1.9	130.5
PAGN	Child-Pugh A	37.7	3.9	5.0	335.1
	Child-Pugh B	38.1	4.0	7.5	466.99
	Child-Pugh C	43.1	5.3	4.0	578.4
	Volunteers	46.3	4.3	7.2	550.9
UCD Subjects (Multiple Dose – PBA Mole Equivalent)					
PBA	Sodium PBA	141.0	2.1	NC	739.0
	HPN-100	70.1	6.1	NC	540.0
PAA	Sodium PBA	53.0	8.1	NC	595.6
	HPN-100	40.5	8.0	NC	574.6
PAGN	Sodium PBA	83.3	7.2	3.9	1133.0
	HPN-100	71.9	8.0	4.8	1098.0

C_{max}= maximum plasma concentration; T_{max} = time of maximum plasma concentration; AUC_{24} = AUC from time 0 to 24 hours; NC = not calculated

[1]Study did not include a sodium phenylbutyrate comparator arm, values represent HPN-100 dosing only. AUC values represent the AUC from time 0 to the last measurable plasma concentration.

[0026] One embodiment of the invention is a method for determining and/or adjusting the dose of ammonia scavenging drugs in patients with UCDs, whereby dose would be based on the amount of dietary protein the patient is consuming, the anticipated percentage conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. Dose adjustments, if necessary, would be based on the observed urinary excretion of PAGN and/or total urinary nitrogen (TUN), the difference between the two reflecting the patient's endogenous capacity for waste nitrogen excretion. This endogenous capacity may be absent in certain patients having innate urea cycle disorders due to inborn metabolic deficiencies,

but patients with later-onset nitrogen accumulation disorders generally have some endogenous capacity, referred to sometimes as their residual urea synthesis capacity. See Brusilow, PROGRESS IN LIVER DISEASES, Ch. 12, pp. 293-309 (1995). The subject's plasma ammonia level may also be determined; this is a critical parameter for tracking effectiveness of an overall treatment program, but reflects a variety of factors such as dietary protein and physiological stress, as well as the effect of a drug used to promote nitrogen excretion.

[0027] Once the patient's residual endogenous capacity for waste nitrogen excretion has been determined, either as the difference between PAGN output and total nitrogen output or as total urinary nitrogen output in the absence of an ammonia scavenging drug, the tolerable amount of dietary protein can be calculated for that patient according to the dosage of the ammonia scavenging drug being administered, or the dosage of the ammonia scavenging drug can be adjusted or calculated to compensate for an estimated protein intake.

[0028] Another embodiment is a method for determining and adjusting the dose of an ammonia scavenging drug to be administered to a patient with liver disease, including hepatic encephalopathy, whereby the starting dose would be based on the amount of dietary protein the patient is consuming, the anticipated conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. While the urea synthetic capacity in patients with liver disease would generally be greater than for patients with UCDs, considerable patient to patient variability would be expected among both groups depending, respectively, on the severity of their liver disease and the severity of their inherited enzymatic defect. Dose adjustments based on the observed urinary excretion of PAGN and total waste nitrogen would adjust for these individual patient characteristics.

[0029] Another embodiment is a method for determining or adjusting allowable dietary protein in the diet of a patient with UCD or with hepatic encephalopathy, who is being treated with an oral PAA-forming ammonia scavenging drug, whereby the amount of allowable protein would be determined by the amount of PAGN and total nitrogen in the urine. The difference between total waste nitrogen in the urine and the amount of PAGN excreted is indicative of the patient's endogenous waste nitrogen processing capacity. Once the patient's endogenous nitrogen processing capacity is known, the patient's endogenous nitrogen processing capacity can be used to adjust dietary protein intake while administering a fixed dosage of an ammonia scavenging drug, or the dosage of the ammonia scavenging drug can be determined according to the amount needed to facilitate elimination of the waste nitrogen from the patient's dietary protein. Dietary protein intake should be determined or adjusted according to how much nitrogen the subject can eliminate above the amount that is

eliminated as PAGN, which results from the PAA-forming ammonia scavenging drug being administered. When making these calculations or adjustments, it is suitable to assume that about 47% of nitrogen in protein will become waste nitrogen that needs to be excreted in the urine (the amount may be less for growing patients, who retain a greater fraction of ingested nitrogen to support body growth), and that about 16% of protein, on average, is nitrogen (see Brusilow 1991).

[0030] It has generally been assumed for such determinations that a prodrug would be converted with 100% efficiency into PAGN for elimination [see, e.g., Berry et al., J. Pediatrics 138(1), S56-S61 (2001) where Figure 1 assumes 100% conversion]; and one report found that about 80-90% of PAA or PBA was excreted from a specific individual as PAGN. Brusilow, Pediatric Research 29(2), 147-150 (1991). It has now been found that HPN-100 and phenylbutyrate are both converted into urinary PAGN at an overall efficiency of about 60% to about 75% on average (about 60% conversion efficiency was seen in UCD patients and about 75% conversion was seen in cirrhotic patients, for example); consequently, this efficiency factor can be used to more accurately calculate or determine initial dosing levels for these drugs, or dietary protein levels acceptable for patients who use these drugs. Given this conversion rate, each gram of HPN-100 can facilitate elimination of waste nitrogen from about a gram (~1.3 grams) of dietary protein per day. Note that PAGN carries away two molecules of ammonia per molecule of PAGN. Examples of calculations based on these parameters are provided in Examples 9 and 10 herein.

[0031] In one aspect, the invention provides a method for transitioning a patient from phenylacetate or phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate, and is adjusted according to the levels of excreted PAGN that result when the prodrug is administered.

[0032] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of ammonia scavenging during the transition (e.g. for clinically 'fragile' patients with a propensity for frequent hyperammonemia). The methods can use two, three, four, five, or more than five steps as judged clinically prudent. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate, amount (i.e. the amount necessary to deliver an equimolar

amount of PBA) of HPN-100 or other prodrug of phenylbutyrate, e.g., if the transition is to be done in three steps, about one-third of the phenylbutyrate would be replaced with a prodrug at each step.

[0033] Another embodiment of the invention is based on observations that delivery of PBA in the form of a glyceryl tri-ester or other prodrug imparts slow release characteristics that allow greater flexibility in dosing schedule. Sodium phenylbutyrate (sodium PBA), for example, is typically dosed every 4 to 8 hours, or even more frequently, in order to maintain a suitable plasma level of PAA. This regimen reflects the rapid absorption of phenylbutyrate from the gastrointestinal tract and quick metabolic conversion to PAA. HPN-100, by contrast, which is a glyceryl tri-ester of phenylbutyrate, has been found to be absorbed only 40% as rapidly as sodium PBA, enabling dosing three times daily, such as with meals, or even twice daily, such as morning and evening. This dosing flexibility is further enhanced by the fact that the pharmacokinetic (PK) and pharmacodynamic (PD) properties of HPN-100 are indistinguishable in the fed or fasted states. It is thus not critical for the frequency of administration to be rigidly maintained with the PBA prodrugs in the form of an ester; the number of doses per day can be reduced for greater convenience, and the dosages do not have to be linked to meal schedules as is recommended in the label for sodium PBA. Indeed, pharmacokinetics for utilization of HPN-100 were very similar when HPN-100 was taken with food or without food, after a day of fasting, so HPN-100 can be taken with food or without food. This translates into a more convenient treatment protocol and potentially higher patient compliance upon substituting HPN-100 for phenylbutyrate or phenylacetate. Surprisingly, even though HPN-100 and sodium PBA are both prodrugs of PAA, HPN-100 is effective when administered less frequently than sodium PBA. While it is typically necessary to administer smaller doses of sodium PBA 3-6 times per day to maintain a stable level of plasma ammonia, similar results can be achieved with only 2-3 doses of HPN-100 per day. In some embodiments discussed in greater detail below, HPN-100 is administered in two doses per day (BID), and in some embodiments it is administered in three doses per day (TID).

[0034] It has also been found that because of the slow-release characteristics of HPN-100, a patient taking HPN-100 has more sustained and often lower plasma levels of PBA and PAA than a patient taking sodium PBA itself. This is believed to be consistent with the greater flexibility in dosing that is discussed in more detail elsewhere in this application (plasma levels of PBA rise and fall more quickly after administration of sodium PBA than after administration of HPN-100).

[0035] Other aspects of this invention relate to the observation that there is apparently no saturation in the ability of the body to convert sodium PBA or HPN-100 to urinary PAGN over a several-fold dose range up to and including, the maximum doses of sodium PBA recommended to date. This should enable a patient to take a higher dose of HPN-100 than an equimolar amount compared to the patient's dosage of PBA. It suggests a patient can receive a higher dosage of HPN-100 than those dosages of sodium PBA that have been recommended to date, which is especially useful for patients whose ammonia levels were not adequately controlled by the highest labeled dosages of sodium PBA. Such patients can receive doses of HPN-100 that are higher than previously recommended sodium PBA dosages.

[0036] Other aspects of the invention will be apparent from the following detailed description and the examples provided herein.

[0037] For convenience, the amounts of PAA (phenylacetic acid), PBA (phenyl butyric acid), or HPN-100 to be administered to a subject as discussed herein refer to a total daily dosage. Because these compounds are used in relatively large daily amounts, the total daily dosage may be taken in two, three, four, five, or six, or more than six daily doses, and different drugs may be administered on different schedules. Thus the total daily dosage better describes a treatment regimen with one drug for comparison to treatments with related drugs.

Brief Description of the Drawings

[0038] Figure 1 shows waste nitrogen disposal via the urea cycle and by the auxiliary pathway involving PAGN.

[0039] Figure 2 depicts a conventional model to describe pharmacokinetic (PK) behavior of a prodrug, which, in the case of phenylbutyrate, assumes that PBA and PAA must reach the systemic circulation in order to be active; i.e., in order to be converted to PAGN and effect ammonia scavenging.

[0040] Figure 3 depicts an adapted model to describe PK behavior of sodium PBA or other drugs such as HPN-100 that can be converted to PBA and PAA, informed by the observations described herein showing that metabolism of HPN-100 results in lower plasma levels of PAA and PBA while providing equivalent pharmacological effect. Unlike the conventional model, this model allows for 'pre-systemic' conversion of PBA/PAA to PAGN and explains inconsistent relationship between blood levels of these metabolites and PAGN-mediated excretion of waste nitrogen

[0041] Figure 4 shows how plasma levels of PAA, PBA, and PAGN change over time following administration of a single dose of either PBA or HPN-100. It shows that the peak level of PAA is lower when the PBA prodrug, HPN-100, is used, and the PAA level at 24 hours post-administration is higher with the prodrug. Thus the prodrug provides a more sustained level of plasma PAA.

[0042] Figure 5 presents data on ammonia levels from the tests in Example 3.

[0043] Figure 6 presents an anatomic explanation for the observations that the prodrug (PBA) can be converted to PAGN prior to reaching the systemic circulation (corresponds to the model depicted in Figure 3).

[0044] Figure 7 shows that PBA levels fluctuate relatively rapidly after dosing in healthy adults, while PAA and PAGN levels reach a fairly stable state after a few days of treatment with sodium phenylbutyrate.

[0045] Figure 8 shows that PBA, PAA and PAGN levels reach steady states at different times in healthy adults and that PAA takes longer to reach a steady state level in cirrhotics

[0046] Figures 9a, 9b, and 9c show that in subjects treated with HPN-100, there is little or no correlation between the dose of HPN-100 and plasma levels of either PBA or PAA in the subject. However, it also shows that urinary excretion of PAGN correlates well with dosage of HPN-100.

[0047] Figure 10 shows plasma ammonia levels [time-normalized area under the curve, or TN-AUC or Area under the curve (AUC)] during the day and night for 10 UCD patients treated for seven days with either sodium PBA or an equimolar dosage of HPN-100, and illustrates that HPN-100 provided better control of ammonia levels than PBA: both the AUC (area under the curve), which is an index of total ammonia exposure, and Cmax, which measures the peak concentration of ammonia, were lower in subjects receiving HPN-100 than in subjects receiving an equimolar dosage of PBA.

[0048] Figure 11 shows that HPN-100 did a better job than PBA of managing plasma levels of nitrogen overnight.

[0049] Figure 12 demonstrates that in patients whose ammonia levels were well controlled on sodium PBA, HPN-100 maintained control. By contrast, patients whose ammonia levels were elevated despite treatment with sodium PBA exhibited the greatest benefit in terms of improved ammonia control from HPN-100.

[0050] Figure 13 summarizes the data from Figure 12 and provides a statistical comparison of ammonia levels for patients on sodium PBA and those on HPN-100. It also shows the normal range for each set of patients.

Modes of Carrying Out the Invention

[0051] In one aspect, the invention is reduced to practice in determining the dose, dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy. The starting dose and schedule would be based upon the theoretical considerations including the estimated percentage conversion of the drug to PAGN, the waste nitrogen resulting from the patient's dietary protein and the percentage of drug converted to and excreted as PAGN. Following initiation of treatment, further dose adjustments would then be made if necessary, upon the actual measurement of urinary PAGN output, or a well-correlated parameter like total urinary ammonia or the ratio of PAGN to creatinine.

[0052] In another aspect, the invention provides a method to transition a patient from phenylbutyrate or phenylacetate to a prodrug of phenylbutyrate (which is a prodrug of PAA), such as HPN-100, or other ester or prodrugs such as compounds of Formula I and II as shown herein. For a number of reasons, HPN-100 is considered a more desirable drug than sodium PBA for many patients who have high ammonia levels and require treatment with an ammonia scavenging drug. In particular, it avoids the unpleasant taste associated with sodium PBA, and it reduces potentially harmful sodium intake, since phenylbutyrate is administered as a sodium salt. A large majority of patients (nine out of ten UCD patients who participated in the clinical study described in example 3) preferred HPN-100 over sodium PBA in clinical testing. Thus many patients who have been treated with phenylbutyrate as an ammonia scavenging drug may want to transition from it to HPN-100.

[0053] It would seem logical for a physician to transition a patient from phenylbutyrate to a prodrug of phenylbutyrate by calculating the amount of the prodrug that would produce an amount of PBA that corresponds to the dosage of phenylbutyrate previously administered to the patient. This would be expected to produce about the same blood plasma level of the active ingredient, PBA. Efficacy of the new treatment with the prodrug could then be assessed by monitoring levels of phenylbutyrate in the blood, to establish the same levels achieved when PBA was administered. As discussed below, however, that approach is not appropriate because, surprisingly, plasma levels of PBA do not correlate well with administered dosages of HPN-100 or with the effectiveness of a dose of HPN-100 or sodium PBA. (Note that sodium PBA is the acid form of phenylbutyrate, which is the common name for the drug BUPHENYL®, and is typically administered as BUPHENYL®, which is a

sodium salt of PBA. References to treatment with PBA herein encompass administration of the phenylbutyrate neutral compound or a salt of phenylbutyrate. Typically, and in all of the working examples herein, PBA is administered as BUPHENYL®.)

[0054] Alternatively, since PBA is a prodrug for PAA, the dosage of a phenylbutyrate prodrug could be calculated according to the theoretically formed amount of PAA, which should be the same amount as what would be calculated from the PBA dosage, since one molecule of PBA is expected to produce one molecule of PAA. The molecular weight of sodium PBA, the registered drug form of PBA (the sodium salt of PBA), is 186; the molecular weight of HPN-100 is 530, and of course HPN-100 provides three equivalents of PBA per molecule, so only one-third as many moles of HPN-100 would be needed to replace a molar quantity of either PBA or PAA. Thus each gram of sodium PBA could be replaced by 0.95 grams of HPN-100; and since HPN-100 is a liquid having a density of 1.1 g/mL, each gram of sodium PBA would be replaced by 0.87 mL of HPN-100, assuming HPN-100 is used as an undiluted liquid. This can be used to select a starting dosage of HPN-100 for patients being transitioned from sodium PBA to HPN-100. Alternatively, a starting dose of HPN-100 in a patient not already taking BUPHENYL® (sodium phenylbutyrate) would need to take into account the surprising observation described in more detail below (see examples 2 and 3) that conversion of the PBA, when administered as HPN-100, into urinary PAGN is incomplete and averages about 60-75%.

[0055] Alternatively, the physician could measure plasma levels of either PBA or PAA in a subject receiving an effective amount of PBA, and determine a dosage of a PBA prodrug by administering enough of the prodrug to produce the same plasma levels of PBA or PAA. The physician could then monitor the amount of either PBA or PAA in the blood to ensure that the appropriate amount of active drug was being produced in the body. It might be expected that a prodrug of phenylbutyrate would provide a slightly lower blood plasma concentration of PAA or PBA than phenylbutyrate, and thus a lower nitrogen-scavenging effect, since conversion of the prodrug to the active drug might be less than 100% efficient. Thus monitoring PAA or PBA plasma levels and increasing the prodrug dosage to bring levels up to those obtained by administering phenylbutyrate might be expected to produce the same physiological effect as the phenylbutyrate dosage. However, it was found that it is not necessary for the plasma level of PAA or PBA observed upon administration of a prodrug of phenylbutyrate to match that produced by an effective

amount of phenylbutyrate, in order to achieve the same ammonia-scavenging effect. Rather, efficacy of the prodrug HPN-100 correlates with urinary PAGN levels, not with plasma levels of PAA or PBA.

[0056] Models have been developed to describe how ammonia-scavenging drugs or prodrugs are expected to behave *in vivo*. One model, shown in Figure 2, reflects conventional approaches to assessing drug effectiveness as applied to HPN-100 based on blood levels of PAA or PBA. Clinical testing has shown that HPN-100 does not produce the plasma levels of PAA and PBA that might be expected from this model, though, even though it is at least as effective on an equimolar basis as PBA for controlling blood ammonia levels, and for eliminating ammonia as PAGN via the urine. Thus the conventional model fails to account for some important metabolic differences between PBA and HPN-100. It was hypothesized that, as compared with sodium PBA, a greater percentage of PBA derived from HPN-100 is converted into PAGN for elimination (or PAA or PBA derived from it) before entering the systemic circulation (the "central compartment" in Figure 2). Recognition of this important and unexpected difference underlies certain aspects of the present invention.

[0057] A refined working model based upon the observations described herein and as outlined in this disclosure is depicted in Figure 3. It supports the conclusion that PBA derived from HPN-100 as well as from sodium PBA can be converted into PAGN without entering into systemic circulation; presumably, HPN-100 or its initial metabolic products (*e.g.*, a compound of formula I wherein one or two of R_1-R_3 represent phenylbutyryl groups, and the remaining one or two of R_1-R_3 represent H—the expected products of partial hydrolysis of HPN-100) may reach the liver and be converted into PAGN there, prior to reaching the systemic circulation. Moreover, the fractional conversion of PBA derived from HPN-100 is greater than for PBA absorbed when PBA is administered as the salt, an observation which explains the lower blood levels of PBA following administration of HPN-100 as compared with sodium PBA despite equivalent or potentially superior ammonia scavenging activity. This observation led to the recognition that plasma levels of PAA or PBA are not reliable indicators of the effectiveness of a PBA prodrug like HPN-100, and should not be relied upon to set or adjust dosages of such PBA prodrug compounds. Data presented herein, e.g. as summarized in Figure 9, demonstrate this effect. Alternative methods for monitoring a subject treated with HPN-100 are needed, and are provided herein.

[0058] In addition, PK/PD modeling, as reflected by considerations and depicted in figures 3 and 6, demonstrate that HPN-100 is absorbed only about 40% as rapidly as PBA

when dosed orally. As a result, HPN-100 provides a slow-release delivery effect, even though it appears to metabolize to PBA rapidly once absorbed. This provides greatly flexibility in dosing and explains why HPN-100 can be dosed, e.g., three times per day or even twice per day to provide similarly stable ammonia levels that require four or more doses of PBA to achieve.

[0059] In view of these observations of unexpected pharmacokinetic behavior, plasma PAA and PBA levels should not be used to evaluate or monitor treatment of a subject with HPN-100 or sodium PBA. Alternative methods are needed, and are provided herein, for monitoring a subject treated with HPN-100. For one, it has been found that between 50 and 85% of HPN-100 is converted into urinary PAGN, typically about 60% to about 75%. This conversion efficiency for HPN-100 and sodium PBA in UCD patients is surprising in light of previous references that have generally assumed the conversion efficiency of sodium PBA to be about 100%. Urinary PAGN has been shown to be inversely correlated with levels of waste nitrogen, e.g. ammonia, in the blood, thus efficacy of HPN-100 can be evaluated by measuring urinary PAGN. It has also been found that HPN-100 has little to no effect on creatinine levels. Moreover, because creatinine levels in healthy adults and patients with nitrogen retention states are typically rather stable, either measuring PAGN output in urine over time, or measuring the ratio of the concentrations of PAGN to creatinine, which can be conveniently done in spot testing, provides a way to monitor HPN-100's effectiveness. In one aspect, the invention thus provides a method to assess the effectiveness of a treatment with HPN-100, comprising determining the ratio of PAGN to creatinine in a 'spot urine' test. Clinical studies show that urinary excretion of PAGN, and the ratio of PAGN to creatinine in urine, correlate well with blood ammonia levels: an increase of PAGN or of the PAGN / creatinine ratio correlates with decreasing plasma ammonia levels. Accordingly, in one method, HPN-100 treated patients are monitored by measuring urinary PAGN output, or by measuring the ratio of PAGN to creatinine in spot urine testing. This method can be used to monitor treatment of a treatment-naïve patient, or of a patient being transitioned from PBA to HPN-100, or a patient being treated with HPN-100. Increasing levels of urinary PAGN output, or an increase in the ratio of PAGN to creatinine in spot testing provides a way to determine whether a dosing regimen that utilizes HPN-100 or another PBA prodrug is promoting elimination of excess ammonia, and to compare two treatment methods to determine which is more effective for the particular subject.

[0060] While plasma ammonia levels are often used to assess disease control in UCD patients, it is often inconvenient to rely upon plasma ammonia levels for optimizing the dosing of HPN-100 outside of a clinical setting. Moreover, plasma ammonia levels are affected by many factors and might be elevated regardless of how well a drug treatment works; it reflects dietary and other factors as well as the adequacy of a drug dosage being used. Plasma ammonia varies a good deal even when relatively well-controlled, based on meal timing, drug timing, and various other factors. Thus to meaningfully reflect drug effect, the plasma ammonia levels need to be monitored over time by repeated blood samplings, which is not practical for routine monitoring of some patients and which does not provide direct information about whether an ammonia scavenging drug is working. Measurements of urinary PAGN, on the other hand, can be done more conveniently as a routine monitoring method because they do not require medical assistance to collect the samples for testing. Moreover, urinary PAGN specifically measures the waste nitrogen clearance provided by the scavenging agent, while many other factors affecting ammonia levels may cause ammonia control to be misleading with regard to the actual effect of the nitrogen scavenging drug. Thus, even though in theory a number of different parameters could be measured to assess effectiveness of a dosage of HPN-100, only measurements based on urinary PAGN are both convenient and reliable as a direct measurement of the nitrogen scavenging drug's effect.

[0061] Thus in one embodiment, the invention provides a method to monitor the effectiveness of treatment of a UCD patient with HPN-100, where monitoring consists essentially of monitoring the patient's urinary PAGN excretion, and optionally checking plasma ammonia levels. Urinary PAGN levels comparable to those achieved with a previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level of less than about 40 μmol/L, or of not greater than 35 μmol/L would indicate the treatment was effective. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test.

[0062] In another aspect, the invention provides a utilization efficiency factor for HPN-100 or for sodium PBA of about 60% to about 75%, which can be used to more accurately determine an initial starting dose of either drug and/or correlate dietary protein intake with projected urinary PAGN.

[0063] In one aspect, the invention provides a method for transitioning a patient from phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method

involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylbutyrate. For example, the amount of HPN-100 needed to provide an equal molar amount of PBA would be calculated (an equimolar amount), and this equimolar amount would be administered to the patient. Urinary excretion of PAGN or plasma ammonia levels would be monitored, and the dosage of HPN would be increased or decreased as needed to establish a level of PAGN excretion that is about the same as that provided by a previously used effective amount of phenylbutyrate or another nitrogen scavenging drug. Typically, a subject being transitioned from PAA or another PAA prodrug onto HPN-100 using this method would be tested for urinary PAGN output prior to the transition and afterwards, and the dosage of HPN-100 would be adjusted as needed to match the urinary PAGN output from this patient when treated with the previous PAA drug or prodrug, assuming the previous PAA prodrug treatment was considered effective. This provides a safer and more effective transition to the new prodrug than methods that rely upon using an equimolar amount without monitoring the *in vivo* effects of that amount of the new drug. It also avoids the risk of inaccurate dosing and potential overtreatment that could result if one monitored PAA or PBA and tried to adjust the prodrug (i.e. HPN-100) dosage to match the PAA or PBA level to the corresponding level provided by administering sodium phenylbutyrate itself.

[0064] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of ammonia scavenging during the transition. In some embodiments, a patient taking an initial dosage of phenylbutyrate is transitioned from phenylbutyrate to a prodrug of phenylbutyrate in steps. The methods can use two, three, four, five, or more than five steps. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate amount of HPN-100 or other prodrug of phenylbutyrate. The appropriate amount for each step can be approximately an amount sufficient to provide an equal molar amount of PBA if it is assumed that the prodrug is quantitatively converted into PBA. Note, too, that BUPHENYL® (sodium phenylbutyrate) contains about 6% inactive ingredients, so it is appropriate to base calculations upon the PBA content of the drug rather than on the weight of the formulated drug. The patient is then monitored to determine how much ammonia scavenging effect has been provided. The amount of HPN-100 (or prodrug) can then be

adjusted to produce about the same amount of ammonia excretion in the form of excreted PAGN that was achieved by the initial dosage of phenylbutyrate, if the patient was well controlled.

[0065] A physician who is switching a patient from PBA to HPN-100 or another ester of phenylbutyrate should be aware that an effective amount of HPN-100 does not necessarily produce a PAA or PBA level that is as high as those seen when sodium phenylbutyrate is administered. It is reported that PAA exhibits some toxicity at high plasma concentrations. Thibault, et al., *Cancer Research*, 54(7):1690-94 (1994) and *Cancer*, 75(12):2932-38 (1005). Given this, and given the unique properties of HPN-100 described above, it is particularly important that a physician not use plasma levels of PAA or PBA to measure the efficacy of HPN-100. If one administers HPN-100 in amounts sufficient to match the plasma PBA or PAA levels provided by administering phenylbutyrate, for example, the dose of HPN-100 may be unnecessarily high.

[0066] The treatment-naïve patient is one not presently receiving an ammonia-scavenging drug treatment to manage nitrogen levels. While there are recommended dosage levels for the nitrogen scavenging drugs in many cases, the right dosage for a naïve patient may be lower than those ranges, for example, and, less commonly, it may be above an equimolar amount when compared to the dosages recommended for sodium PBA. The initial dosage of PAA or a PAA prodrug can be calculated by methods known in the art once a patient's dietary intake of protein is known, and assuming the patient has a relatively normal liver function. Saul W Brusilow, "Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion," *Pediatric Research* 29:147-150, (1991). Methods are also know for measuring the total amount of nitrogen excreted in the urine; in the case of a subject taking a drug that acts by providing PAA, the total waste nitrogen will include PAGN excreted.

[0067] It is estimated that about 47% of nitrogen in proteins consumed will be converted into waste nitrogen, and that about 16% of protein on average is nitrogen. Using these figures, and assuming HPN-100 is efficiently converted to PAGN, a daily dosage of about 19 g of HPN-100 would provide a vehicle to excrete the waste nitrogen from about 43 g of dietary protein; each gram of HPN-100 would thus be able to carry away waste nitrogen from about 2 g of dietary protein. In addition, if it is estimated that HPN-100 utilization efficiency is between about 50% and 85% in various individual patients (as disclosed herein, it has been found that about 60-75% of HPN-100 is converted into urinary PAGN on average), which is consistent with clinical observations to date, and these factors can be

used to further refine the relationship between dietary protein intake and HPN-100 dosing levels for a given subject. With this refinement, each gram of HPN-100 would assist with removal of waste nitrogen for about 1 gram (~ 1.3 grams) of dietary protein. This factor can be used to calculate a suitable dosage of HPN-100 if dietary protein intake is known or controlled, and it can be used to calculate a tolerable dietary protein intake for subject receiving HPN-100.

[0068] This method can also be used to establish a recommended daily dietary protein intake for a patient, by determining the patient's endogenous nitrogen elimination capacity, calculating an amount of dietary protein that this endogenous capacity permits the patient to process without assistance from a nitrogen scavenging drug, and adding to the amount of dietary protein the patient can process on his/her own an amount of protein that the patient would be able to process when using a particular dosage of PBA or a PBA prodrug like HPN-100. Using HPN-100 as an example, a maximum daily dosage of about 19 grams of HPN-100, utilized at an estimated efficiency of 60%, would enable the treated patient to eliminate waste nitrogen corresponding to about 40 g of dietary protein. Thus the invention provides a method to establish a suitable dietary protein level for a patient having a urea cycle disorder or HE, by adding this amount of protein to the amount the patient's endogenous nitrogen elimination capacity can handle.

[0069] In some embodiments, it is also useful to measure PAGN excretion, which accounts for some of the total waste nitrogen excreted when PAA or a PAA prodrug is working. The total waste nitrogen excreted minus the amount of PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via the urea cycle or other mechanisms, and is helpful in determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific. Dosage of HPN-100 can then be established by determining the subject's endogenous capacity to eliminate waste nitrogen; subtracting the amount of dietary protein corresponding to the subject's endogenous nitrogen elimination capacity; and providing a dosage of HPN-100 sufficient to permit the subject to handle the balance of waste nitrogen, based on the subject's dietary protein intake.

[0070] The plasma or blood level of ammonia is optionally also determined, in addition to measuring urinary PAGN, to assess the effectiveness of the overall drug and dietary regimen for a particular patient. If the ammonia control is inadequate, the dosage of the

nitrogen scavenging drug may need to be increased if that can be done, or the patient's dietary protein intake can be decreased if that is feasible.

[0071] In some instances, the dosage of HPN-100 may be limited to dosages that do not exceed recommended dosing levels for phenylbutyrate, adjusting for the fact that each mole of HPN-100 can produces three moles of phenylbutyrate. The label for the use of sodium PBA for the chronic treatment of UCDs recommends a daily dosage not to exceed 20 g; a daily dosage in a range of 9.9-13.0 g/m^2 set according to the subject's size for subjects over 20 kg in weight; and a dosage within a range of 450-600 mg/kg for subjects weighing less than or equal to 20 kg is indicated. While lower doses of HPN-100 may provide comparable ammonia scavenging to PBA on a molar equivalent basis, it may be suitable to select a higher dosage of HPN-100 to achieve adequate ammonia control for certain subjects. Typically, that dose will not exceed the recommended ranges for dosages of phenylbutyrate for a given indication. Thus it may be appropriate to administer HPN-100 at a daily dosage not to exceed an amount of HPN-100 that corresponds to the molar amounts of phenylbutyrate described above (and correcting for the fact that HPN-100 can provide three molecules of PBA). For a subject weighing more than 20 kg, a dosage range for HPN-100 would be between 8.6 and 11.2 mL/m^2. For a subject weighing less than 20 kg, a dosage range of about 390 to 520 μL/kg per day of HPN-100 would be appropriate, based on the use of an equimolar amount compared to the recommended doses of HPN-100. There is no evidence to suggest that HPN-100 would produce adverse effects at a rate in excess of that from an equimolar amount of sodium PBA, so the daily recommended upper limit of 20 g per day of sodium PBA suggests that a daily dose limit of HPN-100 based on the recommendations for sodium PBA would correspond to an equimolar amount of HPN-100, or about 19 g or 17.4 mL.

[0072] Thus in one embodiment, the invention provides a method to monitor the effectiveness of a treatment of a UCD patient with HPN-100, where monitoring consists of, or consists essentially of, monitoring the patient's urinary PAGN excretion and/or plasma ammonia levels. Urinary PAGN levels comparable to those achieved with a previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level that was normal, e.g., a level of less than about 40 μmol/L, or of not greater than 35 μmol/L, would indicate the treatment was effective. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test.

[0073] However, it has also been found that HPN-100 exhibits no indications of toxicity at equimolar doses when compared to the approved PBA dosage of 20 g / day and a dose 2-3 times the equivalent of 20 grams of PBA is unlikely to produce PAA blood levels leading to AEs. Moreover, tolerability of taking HPN-100 is much higher than for PBA and a linear relationship has been observed between HPN-100 dose and PAGN output up to doses of 17.4 mL. In some patients or clinical settings, HPN-100 doses well above the approved PBA dosage are expected to be beneficial; for example, in UCD patients who exhibit recurrent hyperammonemia even on maximal doses of sodium PBA, in UCD patients who need increased dietary protein to support body requirement, or in patients with other nitrogen retaining states.

[0074] Thus in another embodiment, the invention provides methods to treat a subject having HE or UCD, with a dosage of HPN-100 that corresponds to between 100 and 300% of the equimolar amount of the recommended highest dose of PBA. In some embodiments, the suitable dosage will be between about 120% and 180% of the highest recommended dose of PBA; in other embodiments it will be between 120-140% or from 140-160% or from 160-180% of the equimolar amount of the recommended highest dosage of PBA. In accordance with this aspect, the daily dosage of HPN-100 could be as much as 57 g, or up to about 38 g, or up to about 33 g, or up to about 30g, or up to about 25g.

[0075] In one aspect, the invention provides a method to identify the starting dose or dose range and to individually adjust the dose or dose range of a nitrogen scavenging drug comprising PAA or a PAA prodrug (including HPN-100) used for the management of a treatment-naïve patient, which method comprises the steps of:

a) administering an initial dosage of the drug estimated according to the patient's dietary protein load, taking into account the expected percentage conversion to PAGN

b) measuring the amount of total waste nitrogen excreted following administration of the nitrogen scavenging drug comprising PAA or a PAA prodrug;

c) measuring blood ammonia to determine if the increase in urinary excretion of total waste nitrogen is sufficient to control blood ammonia levels; and

d) adjusting the initial dosage to provide an adjusted dosage of the nitrogen scavenging drug comprising PAA or a PAA prodrug based upon ammonia control, dietary protein, and the amount of total waste nitrogen excreted by the patient, or the amount of waste PAGN excreted. Either or each of these parameters can be monitored to assess the dosage of HPN-100 or other nitrogen scavenging drug being administered. Optionally, the

method also includes determining the subject's endogenous nitrogen eliminating capacity (residual urea synthesis capacity) to further help determine an initial dose of HPN-100.

[0076] The initial dosage of the HPN-100 for a treatment naïve patient can be calculated as the amount of waste nitrogen that needs to be eliminated based on the patient's dietary protein intake. This amount can be reduced by an amount equivalent to the waste nitrogen the patient can eliminate using the patient's endogenous waste nitrogen elimination capacity, which can be measured as described herein. The suitable starting dose of HPN-100 can be calculated by estimating dietary protein intake that needs to be managed via the nitrogen scavenging drug, and providing a dose of drug amounting to about 1 g of HPN-100 per 1-2 grams of dietary protein in excess of the amount the patient's endogenous nitrogen elimination capacity can handle, taking into account the expected percentage conversion of the administered PBA to urinary PAGN. The method optionally further includes assessing urinary PAGN output to see if it accounts for the expected amount of waste nitrogen, and optionally may include measuring plasma levels of ammonia in the subject to ensure that an acceptable level of ammonia has been achieved. Checking the patient's plasma ammonia levels provides a measure of the effectiveness of the overall treatment program, including diet and drug dosing.

[0077] The table below summarizes the amount of dietary protein that doses of HPN-100 below (dose 1), within (dose 2) and above (dose 3) those corresponding to the recommended dosages of sodium PBA would be expected to 'cover' (i.e. mediate resulting waste nitrogen excretion), given the following assumptions: 1 gram of PAA mediates the excretion of ~0.18 grams of waste nitrogen if completely converted to PAGN; 60% of the PAA delivered as the PBA prodrug released from HPN-100 is converted to PAGN; 47% of dietary protein is excreted as waste nitrogen, and 16% of dietary protein consists of nitrogen (Brusilow 1991; Calloway 1971). These factors can be used when relating dietary protein intake, drug dosing and waste nitrogen elimination for purposes of the present invention.

HPN-100 Doses and Expected Waste Nitrogen Excretion Based on Dietary Protein

Dose 1	3 mL BID	Corresponds to ~0.47x the dose administered in Example 2, for a 70 kg adult and ~0.35x the amount of PBA (~6.1 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~8.5 g of dietary protein
Dose 2	9 mL BID	Corresponds to ~1.42x the dose administered in Example 2, for a 70 kg adult and ~01.1x the amount of PBA (~18.2 g) delivered in the maximum

		approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~26 g of dietary protein
Dose 3	15 mL BID	Corresponds to ~2.36x the dose administered in Example 2, for a 70 kg adult and ~1.73 x the amount of PBA (~30.3 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~43 g of dietary protein

[0078] As used herein, plasma levels of ammonia are acceptable when they are at or below a level considered normal for the subject, and commonly this would mean plasma ammonia level is below about 40 μmol/L. In certain clinical tests described herein the upper limit of normal for the subjects was between 26 and 35 μmol/L, and it is recognized in the art that a normal ammonia level will vary depending upon exactly how it is measured; thus as used to describe ammonia levels herein, 'about' means the value is approximate, and typically is within ± 10% of the stated numeric value.

[0079] In other aspects, the invention provides a method to identify a suitable starting dose or dose range for a UCD or HE patient and to individually adjust the dose or dose range of a new nitrogen scavenging drug used for the management of a patient already treated with a previous nitrogen scavenging drug, which method comprises the steps of:

a) administering an initial dosage of the new nitrogen scavenging drug (which can be estimated according to the patient's dietary protein load and/or the dose of the new drug expected to yield the same amount of urinary PAGN excretion as a previously used nitrogen scavenging drug);

b) measuring the amount of total waste nitrogen and/or of PAGN excreted following administration of the new drug;

c) optionally measuring blood ammonia to determine if the initial dosage is sufficient to control blood ammonia levels, or to establish a suitable average ammonia level; and

d) adjusting the initial dosage of the new drug as needed to provide an adjusted dosage based upon ammonia control, dietary protein, and the amount of total waste nitrogen excreted by the patient. The adjusting of the initial dosage is done based on the amount of urinary PAGN, without relying upon plasma levels of PAA, PBA, or PAGN, and preferably without relying upon plasma levels of ammonia.

[0080] Where the patient has previously been treated with PAA or a PAA prodrug, the treating physician may rely, wholly or in part, upon the previous treatment to set a dosage

for a new PAA prodrug, or a PBA prodrug, to be administered to the same patient. If the previous drug was reasonably effective for managing the patient's condition, the physician may set the dosage for a new PAA or PBA prodrug by reference to the previous one, so that the new drug is administered at a dosage that provides the same dosage of PAA to the patient, assuming complete conversion of each prodrug into PAA.

[0081] Again, as discussed above, it is sometimes desirable to measure PAGN excreted in addition to total waste nitrogen excreted. The total waste nitrogen excreted minus the amount of PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms, and is helpful in determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific.

[0082] In another aspect, the invention provides a method to identify the amount of dietary protein that could be safely ingested by a subject with a nitrogen accumulation disorder, including hepatic encephalopathy and UCD, where the patient is taking an ammonia-scavenging drug that comprises PAA or a PAA prodrug, which method comprises the steps of:

a) measuring the amount of total waste nitrogen excreted following administration of the drug,

b) determining the amount of dietary protein calculated to yield an amount of waste nitrogen less than or equal to urinary waste nitrogen; and

c) adjusting dietary protein and/or drug dosage as appropriate based upon measurement of blood ammonia and total waste nitrogen excretion.

[0083] Where the subject is receiving treatment with a nitrogen-scavenging drug, it may be necessary to reassess the patient's dietary intake of protein periodically, since many factors will affect the balance between nitrogen intake, nitrogen excretion, and dosage of a nitrogen scavenging drug. The invention provides methods to determine how much dietary protein a patient can handle, based on measuring the patient's nitrogen excretion levels. It may further be useful to measure the patient's PAGN level as discussed above, to help determine the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms.

[0084] In the above methods, the patient may be one having a urea cycle disorder, or other nitrogen accumulation disorders. In many embodiments, the methods are applicable to patient's having a urea cycle disorder, but relatively normal liver function.

[0085] The above methods can be practiced with a variety of prodrugs of PAA or PBA. In some embodiments, HPN-100 is the PBA prodrug of choice for these methods.

[0086] In another aspect, the invention provides a method to transition a patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the portion of phenylacetate or phenylbutyrate; and

c) monitoring the amount of PAGN excreted by the patient to assess the effectiveness of the replacement amount of the prodrug.

[0087] Optionally, this method comprises adjusting the amount of the prodrug and administering an adjusted amount of the prodrug, then further monitoring PAGN excretion to assess the effectiveness of the adjusted amount of the prodrug. The replacement amount of the PBA prodrug can be about an equimolar amount to the amount of PBA being replaced.

[0088] For reasons discussed extensively herein, it is misleading to rely upon PAA levels when moving a patient to a prodrug (or a new prodrug) of PAA or PBA. The availability of liver-based mechanisms for rapid conversion of a prodrug into PAGN without necessarily entering the systemic system renders plasma levels of PAA and PBA insufficient as predictors of efficacy, so the method relies upon the excreted PAGN for assessing and monitoring treatment with a PAA or PBA prodrug that is to be given to the patient.

[0089] In many cases, it will be possible to transition a patient directly from, e.g., phenylbutyrate to HPN-100 or another PBA prodrug in a single stage, rather than in incremental steps. Thus all of the previously used PAA or PAA prodrug may be replaced with a suitable substitution amount of the new drug (PBA prodrug). However, in some situations (e.g. 'fragile patients', patients taking dosages at or near the recommended limits of PAA or PAA prodrug, and for patients having very limited endogenous capacity for excreting nitrogen wastes, or in situations where the ability of the patient to metabolize or

excrete the drug is uncertain), it may be preferable to transition from the initial drug to a new PBA prodrug like HPN-100 in two or more stages or steps. Thus the transition may be made in 2, 3, 4 or 5 steps, and at each step a fraction of the original drug (e.g, about half for a two-step transition, about a third for a three-step transition, etc.) is replaced by the new PBA prodrug to be administered. This approach might be appropriate for a 'fragile' UCD patient known to be susceptible to repeated episodes of hyperammonemia while receiving treatment or while taking a large amount of drug that promotes nitrogen elimination.

[0090] Thus in another aspect, the invention provides a method to transition a UCD patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the phenylacetate or phenylbutyrate; and

c) monitoring plasma level of ammonia in the patient to assess the effectiveness of the replacement amount of the prodrug.

[0091] In some embodiments, the replacement amount of the prodrug is an equimolar amount compared to the amount of PBA being replaced

[0092] During the monitoring step, the patient is being treated with a mixture of phenylacetate or phenylbutyrate plus the new prodrug. The proportion depends upon what step of the transition the patient is in. The physician can also use information about the effects of a first step in setting the replacement amount of the prodrug for use in subsequent steps; thus if the prodrug is significantly more effective than predicted when the estimated amount used as a replacement amount is administered in a first step, the replacement amount used in a subsequent step of the transition can be proportionally reduced.

[0093] In another aspect, the invention provides a method to initiate treatment with phenylacetate, phenylbutyrate or a PBA prodrug in a step-wise fashion, as might be appropriate for a 'fragile patient' (a UCD patient with a history of frequent symptomatic hyperammonemia and/or neonatal onset disease who presumably has no urea synthetic capacity, or a patient with severely compromised liver function whose ability to metabolize the drug may be uncertain). This process may be more complex, since the prodrug will rely

upon liver function to be activated and to function; thus the method is preferably done in a stepwise fashion, exemplified by the following steps:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as excreted PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

[0094] The methods also include optionally measuring total urinary nitrogen and urinary PAGN after at least 3 days of drug administration, at which point a steady state has been achieved. It also can include calculating the amount of drug converted to PAGN, which would be expected to be at least 50%, to determine if the drug is having the desired effect. A suitable dosage of the drug would be identified as one where the amount of excreted PAGN is sufficient to clear the expected amount of waste nitrogen from the dietary intake of protein, which can be adjusted to account for the patient's endogenous nitrogen elimination capacity.

[0095] The fraction of nitrogen waste to be cleared in a single step can be selected with due regard to the severity of the patient's condition (nitrogen accumulation disorder). In some embodiments, it will be appropriate to target removal of about 50% of the waste nitrogen for which clearance assistance is needed. In some embodiments, the method will target removal of about 100% of the waste nitrogen.

[0096] In another aspect, the invention provides a method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted PAGN for the subject to make sure it has not decreased; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

[0097] If it is found that the amount of excreted PAGN decreases, additional HPN-100 or additional PBA would be administered to reestablish a level of PAGN excretion that is suitable for the patient, and the replacement steps would then be continued until all of the PBA was replaced by HPN-100.

[0098] Here again, the portion of phenylbutyrate to be replaced in an initial step can be 100%, about 1/2, about 1/3, or about ¼, or some value between these. During a stepwise process, where less than all of the phenylbutyrate is replaced in a first step, the patient will receive both HPN-100 and phenylbutyrate. As demonstrated herein, the appropriate method for determining a suitable dose of HPN-100 will take account of the excreted PAGN, rather than being based only on less reliable criteria for evaluating the orally delivered PBA prodrug.

[0099] In another embodiment, the invention provides a method to administer a phenylbutyrate prodrug to a patient, comprising determining the rate of PAGN excretion for the subject following administration of at least one phenylbutyrate prodrug, and selecting or adjusting a dose administration schedule based on the PAGN excretion rate. The compound can be a compound of Formula I, Formula II or Formula III as described above. Advantageously, the compounds used herein as prodrugs of PBA achieve nitrogen scavenging comparable to that of PBA but exhibit a slow-release kinetic profile that produces a more stable ammonia level in the treated subject. In some embodiments, the methods of the invention include administering a prodrug as described herein to a subject at a dosage that provides comparable ammonia level control to that achieved by PBA, but with significantly lower exposure of the subject to systemic PBA. In some embodiments, the subject experiences pharmacokinetic parameters for PBA that demonstrate lower exposure to PBA, including a lower AUC and Cmax for PBA, while maintaining a plasma ammonia level comparable to or better than that provided by treatment with a dosage of PBA within the normal dosing range. When HPN-100 and PBA were administered to UCD patients at equimolar dosages, the patient receiving HPN-100 had overall lower plasma ammonia levels, and also lower PBA exposure:

	AUC (NH_3) μg-hr/mL	C_{max} (NH_3) μg-hr/mL	AUC (PBA) μg-hr/mL	C_{max} (PBA) μg-hr/mL
PBA	38.4(20)	79.1(40)	739(49)	141(44)
HPN-100	26.1(10)	56.3(28)	540(60)	70(65)

[00100] While a larger data set is needed to demonstrate statistical significance, limited amounts of data are available in part due to the rarity of these conditions. Nevertheless, the data indicates that PBA treatment resulted in less effective ammonia level control and greater exposure to PBA, while the PBA prodrug HPN-100 at equimolar dosing provided better ammonia level control and lower PBA exposure levels. Accordingly, in one aspect the invention provides a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces better ammonia level control than PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA, when compared to treatment with an equimolar amount of PBA. In some embodiments, the treatment uses HPN-100 as the prodrug, and in some embodiments the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20%; or the exposure to PBA upon treatment with the prodrug is lower by at least about 30% compared to treatment with PBA; or both of these conditions are met to demonstrate reduced exposure to PBA. In some embodiments, the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the prodrug is administered. Preferably, the prodrug provides plasma ammonia levels that average less than about 40 μmol/L or not more than 35 μmol/L.

[00101] The advantageous slow-release kinetic profile of compounds used herein as prodrugs of PBA permits less frequent and more flexible dosing in selected patients as compared with sodium PBA. While all patients with UCDs and a propensity for elevated ammonia levels should in principle be able to benefit from the ammonia scavenging activity of HPN-100, UCD patients with substantial residual urea synthetic capacity (e.g. UCD whose first manifestations occur at several years of age or older; i.e. patients who do not exhibit neonatal onset) would be the best candidates for three times daily or even twice daily dosing with PBA prodrugs such as HPN-100. Patients with cirrhosis and HE would also be candidates for less frequent dosing, as even patients with severe liver disease have significant residual urea synthetic capacity (Rudman et al., J. Clin. Invest. 1973).

[00102] Specific embodiments of the invention include the following:

A. 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, wherein monitoring the effect consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output.

In this method, the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and urinary PAGN output can be determined as a ratio of urinary PAGN to urinary creatinine, since it has been demonstrated by others that creatinine, the daily excretion of which tends to be constant for a given individual, can be used as a means to normalize measures of urinary parameters while correcting for variations in urinary volume. In these methods, the nitrogen retention disorder can be chronic hepatic encephalopathy or a urea cycle disorder. Plasma ammonia levels may also be monitored to adjust the overall treatment program and dietary protein intake, but as discussed above, urinary PAGN provides a preferred way to assess the drug's role in waste nitrogen elimination.

B. 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, wherein the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and wherein 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. In these methods, administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level in the patient. This can be a level of about 35 or about 40 μmol/L.

C. A method to determine a starting dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a utilization efficiency of about 60% to about 75%. In such methods, the dosage of HPN-100 can be calculated from the patient's dietary protein intake, or it can be estimated from the patient's body weight and approximate growth rate. In such methods, the dosage of HPN-100 is sometimes reduced to account for the patient's residual urea synthesis capacity, by adjusting the amount of HPN-100 to reflect the amount of ammonia scavenging needed in view of the patient's endogenous capacity for nitrogen elimination.

D. A method to determine a dosage of a PAA prodrug for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) 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.

In these methods, the PAA prodrug can be phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof, or it can be HPN-100.

E. A method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) 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; and

e) administering to the patient the suitable dosage of the PAA prodrug.

In these methods, the PAA prodrug is often phenylbutyrate or a pharmaceutically acceptable salt thereof, or HPN-100.

G. A method to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, comprising:

a) determining a replacement amount of HPN-100 to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the HPN-100 for the phenylacetate or phenylbutyrate; and

c) monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the HPN-100.

In these methods, an increase the amount of urinary PAGN may indicate that the amount of HPN-100 can be reduced, and a decrease in urinary PAGN may indicate the amount of HPN-100 needs to be increased.

H. A method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted urinary PAGN for the subject; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

I. A method to initiate treatment with phenylacetate, phenylbutyrate or a HPN-100 in a step-wise fashion, comprising:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion based upon diet and urea synthetic capacity; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as urinary PAGN taking into account the expected percentage conversion of the administered PBA to urinary PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

J. A method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA when the patient receives the PBA prodrug, when compared to the AUC and Cmax observed when the patient receives an equimolar amount of PBA.

In these methods, the PBA prodrug is often HPN-100.

The methods include a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA exposure can be lower with the prodrug than with PBA by at least about 20%, or by at least about 30% compared to treatment with PBA. This is believed to be related to the slow absorption or uptake

characteristics of HPN-100, which provide a more stable level of PBA exposure and provide an unexpected advantage of HPN-100 to be effective with less frequent dosing when compared to sodium phenylbutyrate.

K. A method to determine a suitable dietary protein level for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's endogenous nitrogen elimination capacity;

b) calculating from the endogenous nitrogen elimination capacity an amount of dietary protein the patient can process without the aid of a nitrogen scavenging drug;

c) then adding an amount of protein that the patient should be able to process with the assistance of selected dosage of a nitrogen scavenging drug to arrive at an amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, taking into account the amount of protein required for health and body growth.

In this method, the nitrogen scavenging drug can be HPN-100. Commonly, the selected dosage of HPN-100 is not more than about 19 grams per day, and the amount of dietary protein the patient should be able to process with the assistance of this amount of HPN-100 is about 1 grams (~1.3 g) of protein per gram of HPN-100.

L. A method to treat a patient with a PBA prodrug, comprising administering HPN-100 at a daily dose in excess of 19 g per day to a subject having HE or UCD. Optionally, the daily dose of HPN-100 is between about 20 g and about 57 g.

M. A method for determining the dosing schedule of a PBA prodrug wherein the patient retains substantial residual urea synthetic capacity, as would be the case for most patients with cirrhosis and HE or most UCD patients who do not exhibit symptoms within the first two years of life.

[00103] In the foregoing methods that utilize HPN-100, the exposure to PBA upon treatment with the prodrug HPN-100 is lower by at least about 30% compared to treatment with PBA. Also, commonly the AUC for PBA is less than about 600 and the Cmax for PBA

is less than about 100 when the prodrug is administered. Also, in the foregoing methods, when the subject is treated with the prodrug, which can be HPN-100, the subject will typically achieve and maintain normal plasma ammonia levels.

[00104] The following examples are offered to illustrate but not to limit the invention.

[00105] The data below from three human studies and one preclinical study illustrate that the conventional approach of assessing drug exposure and effect by measuring blood levels does not correlate with nitrogen scavenging as assessed by urinary excretion of PAGN or by reduction of plasma ammonia. These data demonstrate that, surprisingly, the plasma level of PBA or PAA seen with an effective amount of a prodrug can be far less the plasma level of PBA or PAA seen with a similarly effective amount of phenylbutyrate. Moreover, they demonstrate the need to allow for incomplete conversion of sodium PBA or HPN-100 into PAGN in selecting starting dosage, the delayed release behavior and implications for dosing schedule of delivering PBA as a triglyceride rather than as a salt, and the possibility of administering HPN-100 in doses greater than those currently recommended for sodium PBA. These are followed by a biological explanation for the findings.

Example 1

Single dose safety and PK in healthy adults

[00106] To assess its pharmacokinetic (PK) and pharmacodynamic (PD) profile, HPN-100 was administered as a single dose to 24 healthy adults. Pharmacokinetic samples were taken pre-dose and at 15 and 30 minutes post-dose and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours post-dose. As discussed below, plasma levels of the major HPN-100 metabolites PBA, PAA and PAGN were many fold lower after administration of HPN-100 than after sodium PBA. By contrast, urinary excretion of PAGN was similar between the two groups (4905 +/- 1414 mg following sodium PBA and 4130 +/- 925 mg following HPN-100) and the differences that were observed were determined to be largely an artifact of incomplete collection due to stopping urine collection at 24 hours (note that PAGN excretion following administration of sodium PBA was largely complete at 24 hours but continued beyond 24 hours following administration of HPN-100). Thus, the plasma metabolite concentrations did not accurately reflect the comparative ammonia scavenging activity of sodium PBA and HPN-100.

[00107] Three healthy adult volunteers were treated with a single dose of either sodium PBA or HPN-100 at a dosage of 3 g/m^2. Plasma levels of PAA, PBA, and PAGN were

monitored periodically for 12-24 hours by known methods. Results of this are shown in Figure 4, which shows a curve for each subject (note the log scale).

[00108] In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving sodium PBA at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100.

[00109] In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately 12 hours, and were generally close to the PAGN curves up to that time.

Example 2

Administration of HPN-100 to patients with liver disease

[00110] To determine its pharmacokinetic (PK) and pharmacodynamic (PD) profile in patients with liver disease, clinical testing was conducted in which HPN-100 was administered orally as a single dose (100 mg/kg/day on day 1), and twice daily for 7 consecutive days (200 mg/kg/day on days 8 through 14, in two doses of 100 mg/kg per dose), to subjects with hepatic impairment with cirrhosis (Child-Pugh scores of A, B, or C) and to a gender and age-matched control group of healthy adults with normal hepatic function. On day 15, subjects received a single dose of HPN-100 (100 mg/kg). PK blood samples were taken pre-dose, at 15 and 30 minutes post-dose, and at 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose on days 1, 8, and 15, and at 48 hours after dosing on days 1 and 15. On days 9–14, blood samples were taken pre-morning dose and at 2 hours post-morning dose. Urine was collected 0–4, 4–8, 8–12, and 12–24 hours post-dose on days 1, 8, and 15, and at 24–48 hours post-dose on days 1 and 15.

[00111] HPN-100 was metabolized via the predominant pathway in all subject groups, and the alternative HPN-100 metabolites PAG (phenylacetyl glycine), PBG (phenylbutyryl glycine), and PBGN (phenylbutyryl glutamine) were below the limit of quantification in all plasma samples. Both the extent of systemic exposure (AUC_{0-t}) and C_{max} for PBA and PAA tended to be higher in Child-Pugh group B or C than in Child-Pugh group A or the healthy volunteer group, although there were no significant differences in these variables on day 15.

As described below, plasma PAA levels did correlate with Childs-Pugh classification (i.e. were higher in patients with more severe liver disease). However, the average conversion of HPN-100 to PAGN was ~ 75%, and no difference were seen between patients with cirrhosis and normal healthy volunteers, demonstrating that hepatic impairment did not affect the subjects' ability to activate the PBA prodrug HPN-100 or to utilize it for elimination of excess ammonia. Thus, as summarized in more detail below, plasma metabolite levels did not correlate well with the HPN-100 dosage and, just as for healthy adults, plasma metabolite levels did not accurately reflect the nitrogen scavenging effect of HPN-100. Moreover, the mean conversion of administered PAA to PAGN averaged ~75% in this patient population.

Analyte	Subject group	Geometric mean ratio	90% CI	P value for group effect
PBA	**AUC_{0-t}**			0.40
	Child-Pugh A	0.92	0.58–1.43	
	Child-Pugh B	1.26	0.80–1.97	
	Child-Pugh C	1.37	0.87–2.14	
PBA	**C_{max}**			0.52
	Child-Pugh A	1.42	0.87–2.31	
	Child-Pugh B	1.35	0.83–2.21	
	Child-Pugh C	1.50	0.92–2.45	
PAA	**AUC_{0-t}**			0.64
	Child-Pugh A	1.22	0.48–3.06	
	Child-Pugh B	1.53	0.61–3.85	
	Child-Pugh C	1.94	0.77–4.88	
PAA	**C_{max}**			0.72
	Child-Pugh A	1.33	0.70–2.52	
	Child-Pugh B	1.16	0.61–2.20	
	Child-Pugh C	1.52	0.80–2.88	

AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum observed plasma concentration; PAA, phenylacetic acid; PBA, phenylbutyric acid.

[00112] During multiple dosing (days 8–15), there was a trend for higher systemic concentrations of PBA and PAA in subjects with greater hepatic impairment (Child-Pugh B

or C) compared with Child-Pugh group A and the healthy volunteers. Unlike PBA, PAA did accumulate significantly in plasma during multiday dosing. Differences between single (day 8) and multiple dosing (day 15: steady state) were significant for AUC_{0-12} and C_{max} of PAA for all subjects combined ($p < 0.001$), but not for PBA. After dosing on day 15, extent of exposure to PAA, but not PBA, significantly correlated with hepatic impairment.

[00113] The clinical efficacy of HPN-100 is dependent on its ammonia scavenging capabilities, through conjugation of glutamine with PAA to form PAGN. After dosing on each day, PAGN was the major metabolite excreted: 42–49% of the HPN-100 dose administered was excreted as PAGN on day 1, 25–45% on day 8, and 58–85% on day 15. Very low amounts of PBA and PAA were excreted in the urine (≤ 0.05% of the total HPN-100 dose). There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. Urinary PAGN excretion is also an indication of the ammonia-scavenging capacity of HPN-100, as 2 moles of ammonia combine with 1 mole of PAA to produce PAGN. Hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study. There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. The observations that hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study but was associated with accumulation of PAA in plasma underscores the importance of utilizing urinary PAGN rather than metabolite blood levels to guide drug effect and, as a corollary, the importance of the invention, as does the fact that the mean percentage conversion of administered PAA into urinary PAGN among the 4 treatment groups was ~75%.

Urinary PAGN Excretion After Dosing on Day 15 (0-48 Hours).

	Child-Pugh A (8)	Child-Pugh B (8)	Child-Pugh C (8)	Healthy Adults (8)
Amount excreted (µmol) Mean (SD) Range	31431 (15291) 16016–65229	25152 (11426) 13643–41635	30752 (20860) 6331–60139	28716 (8223) 17203–41092
Molar % of dose excreted Mean (SD) Range	79.6 (30.5) 48.9–138.2	58.2 (29.2) 26.5–99.6	85.0 (65.1) 23.1–221.1	68.6 (21.9) 30.6–96.
Molar % of dose ammonia scavenged Mean (SD) Range	159.2 (60.9) 97.9–276.4	116.3 (58.3) 53.0–199.2	169.9 (130.1) 46.3–442.3	137.2 (43.9) 61.3–193.4

[00114] Of particular note, there was no relationship between the plasma levels of PBA and PAA, which exhibited a non-statistically significant directional change toward higher plasma levels in patients with liver disease than healthy adults, and urinary excretion of PAGN.

EXAMPLE 3

Administration of HPN-100 To Adults With UCDs

[00115] To further explore its pharmacokinetic (PK) and pharmacodynamic (PD) profile in clinical states associated with nitrogen retention, 10 adult UCD patients were switched from sodium PBA to a PBA equimolar dose of HPN-100. Subjects were required to be on a stable dose of sodium PBA before enrolment. Upon enrolment, all subjects received sodium PBA for 7 days and were then admitted to a study unit (Visit 2-1) for overnight observation and 24-hour PK and ammonia measurements and urine collections. Subjects were then converted to the PBA equimolar dose of HPN-100, either in a single step or in multiple steps depending on the total dose of sodium PBA; 9 out of 10 patients converted in a single step. Subjects stayed on the 100% HPN-100 dose for one week and were then re-admitted to the study unit for repeated PK (Visit 11-1), ammonia and urine collections.

[00116] The findings from this study, summarized in detail below, demonstrate that, just as in healthy adults and patients with liver disease, plasma metabolite levels do not correlate well with ammonia scavenging activity as reflected by urinary PAGN excretion and

corroborated by plasma ammonia results. Moreover, the findings demonstrate considerable inter-individual variability in the percentage of both sodium PBA and HPN-100 that is converted to urinary PAGN.

[00117] Pharmacokinetic, ammonia and safety analyses: As summarized in the table below, 7 days of HPN-100 administration resulted in comparable PAA and plasma PAGN levels but slightly lower PBA levels compared to the PBA molar equivalent dose of sodium PBA.

Comparison of Pharmacokinetic Parameters at Steady State – sodium PBA vs. HPN-100

PK Parameter	Arithmetic Mean (CV%)	
	Sodium PBA (N=10)	HPN-100 (N=10)
PBA in Plasma		
AUC_{0-24} (μg·h/mL)	739 (49.2)	540 (60.1)
$Cmax_{ss}$ (μg/mL	141 (44.3)	70.1 (64.7)
$Cmin_{ss}$ (μg/mL	0.588 (255)	2.87 (265)
PAA in Plasma		
AUC_{0-24} (μg·h/mL)	595.6 (123.9)	574.6 (168.9)
$Cmax_{ss}$ (μg/mL	53.0 (94.7)	40.5 (147.6)
$Cmin_{ss}$ (μg/mL	3.56 (194.4)	7.06 (310.7)
PAGN in Plasma		
AUC_{0-24} (μg·h/mL)	1133 (31.1)	1098 (44.2)
$Cmax_{ss}$ (μg/mL	83.3 (25.8)	71.9 (56.0)
$Cmin_{ss}$ (μg/mL	16.8 (86.1)	12.1 (134.4)

AUC_{0-24}: Area under the concentration from time 0 (pre-dose) to 24 hours, $Cmax_{ss}$: Maximum plasma concentration at steady state, $Cmin_{ss}$: Minimum plasma concentration at steady state, A_e: Amount excreted over 24 hours

[1] The mean (SD) sodium PBA dose = 12.6 (4.11) g; the mean (SD) HPN-100 dose = 12.3 (3.91) g.

[00118] Despite dissimilar PBA blood levels, overall urinary excretion of PAGN was similar for the two treatments as summarized in the table below. Importantly, and in contrast to the assumptions inherent in current treatment guidelines that all administered sodium PBA is converted to urinary PAGN, considerable inter-individual variability was observed in the percentage of administered PAA converted to PAGN, which averaged ~60% and similar both sodium PBA and HPN-100. Moreover, the 24 hour pattern of excretion appeared to differ in that urine output of PAGN reached its highest level during the 'afternoon hours' (6-12 hour urine collection) for patients treated with sodium PBA, whereas peak output of PAGN occurred overnight (12-24 hour urine collection) for patients

on HPN-100 treatment. This difference presumably reflects the slow release characteristics and longer duration of effective blood concentrations of PAA following administration of HPN-100 as compared with sodium PBA. HPN-100 was either not detectable or below the limits of quantitation in all blood samples.

Comparison of Mean PAGN Amount Excreted (μg) – sodium PBA (sodium phenylbutyrate) vs. HPN-100

Treatment	PAGN 0-6 hours	PAGN 0-12 hours	PAGN 12-24 hours	Total PAGN Excretion (CV%)
sodium PBA	2,452,838	4,859,121	4,645,447	12,153,473 (48.2)
HPN-100	2,381,371	3,027,310	5,433,033	10,784,747 (25.9)

[00119] As summarized in the table below, mean time normalized area under the curve (TN-AUC) values for venous ammonia following HPN-100 were directionally (~31%) lower than those observed with sodium PBA (26.1 vs. 38.4 μmol/L) although the differences did not achieve statistical significance (Figure 10). Likewise, peak venous ammonia concentrations following HPN-100 were directionally (~29%; not statistically significant) lower than those observed with sodium PBA (56.3 vs. 79.1 μmol/L, respectively).

[00120] The normal upper limit for venous ammonia varied among the study sites from 26 to 35 μmol/L. Examination of ammonia values (TN-AUC) for individual patients demonstrated that patients with higher ammonia levels on sodium PBA exhibited greater decreases in ammonia values following administration of HPN-100 (Figure 12). Moreover, the mean ammonia value after HPN-100 (26.1 μmol/L) was within the normal range while it was above the upper limit of normal (ULN) after sodium PBA (sodium phenylbutyrate) (38.4 μmol/L) (Figure 13). Likewise the mean percentage of normal ammonia values increased from 58% after sodium PBA treatment to 83% after HPN-100 treatment.

Venous Ammonia Pharmacodynamics Following Seven Days of Dosing With Either Sodium PBA or HPN-100 (Steady State)

	Sodium PBA			HPN-100		
Subject	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC** (μmol/L)	**PBA Equivalent dose[1]**	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**
1001	29.0	16.47	17.5	63.0	19.8	13.1
1002	31.0	20.9	15.8	31.0	19.3	15.9
1004	85.0	46.8	99.2	106	35.1	9.16
1006	150	71.5	17.5	13.0	8.30	17.7
2001	88.0	52.1	6.57	33.0	22.7	6.71

	Sodium PBA			HPN-100		
Subject	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC** (μmol/L)	**PBA Equivalent dose[1]**	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**
2003	31.0	17.5	11.8	74.0	21.1	12.2
3002	108	22.3	16.5	36.0	21.9	17.7
3004	115	62.9	13.1	75.0	38.4	13.1
5001	82.2	35.8	8.76	57.0	35.5	8.85
5002	72.2	37.7	8.76	75.2	39.1	8.85
N	**10**	**10**	**10**	**10**	**10**	**10**
Mean	79.1	38.4	12.6	56.3	26.1	12.3
SD	40.1	19.6	4.11	27.9	10.3	3.91
Median	83.6	36.8	12.5	60.0	22.3	12.7
Min	29.0	16.4	6.57	13.0	8.30	6.71
Max	150	71.5	17.5	106	39.1	17.7
25%	31.0	20.0	--	32.5	19.7	--
75%	110	54.8	--	75.0	36.2	--

[00121] This reduction in ammonia exposure among UCD patients reflects better overnight control among subjects receiving HPN-100, as summarized in the table below and in Figure 11. This study shows that both AUC and Cmax for ammonia were lower with HPN-100, indicating less total ammonia exposure, and especially at night, HPN-100 exhibited a significantly stronger effect. While not statistically significant due to the small population size, this demonstrates that HPN-100 is at least as effective, and apparently more so, than PBA on an equimolar basis based on the key measure, its ability to mobilize ammonia for urinary elimination. Based on preliminary results, HPN-100 also provides more stable ammonia levels, and reduces risk of hyperammonemia. In this trial, 9 of 10 subjects who experienced both HPN-100 and sodium PBA indicated a preference for HPN-100.

[00122] In addition, in this trial, no serious adverse effects (SAEs) were observed in patients taking HPN-100, while two subjects receiving PBA experienced symptomatic hyperammonemia; and the total number of adverse effects (AEs) reported among subjects taking HPN-100 (5 subjects reported a total of 15 AEs) was lower than the number of AEs among subjects taking PBA (7 subjects reported 21 AEs).

[00123] The following table summarizes overall comparative data for sodium PBA and HPN-100, administered at equimolar rates (n=10) (see tables above and figures 10-13 for additional detail).

Parameter	Sodium PBA	HPN-100
NH_3: Total AUC	38.4 ± 19.6	26.1 ± 10.3
NH_3 Cmax	79.1 ± 40.1	56.3 ± 27.9
NH_3 exposure: DAY (hours 6-12)	37.1	32.9
NH_3 exposure: NIGHT (hours 12-24)	36.3	21.3
Adverse effects	21 reported by 7 subjects	15 reported by 5 subjects
Serious adverse effects	2 (symptomatic hyperammonemia)	0
PAGN excretion	Comparable	Comparable

[00124] While the differences between sodium PBA and HPN-100 did not reach statistical significance due to the small sample size, HPN-100 exhibited a clear trend toward being more efficacious at equimolar dosages, and it was particularly effective for improving overnight control of ammonia levels.

[00125] Figure 9a demonstrates that PBA levels in the blood are not correlated with HPN-100 dosages received. It plots the 24-hour AUC for PBA and the Cmax for PBA against HPN-100 dosage (top panel), and while the AUC and Cmax track together in each patient, they show no relationship to HPN-100 dose: both the highest and the lowest PBA exposures occurred in patients receiving high doses of HPN-100. Figure 9b shows that levels of PAA are similarly uncorrelated with HPN dosages.

[00126] Figure 10 illustrates the trend shown in the clinical testing, where HPN-100 provided better overall control of waste nitrogen.

[00127] Figure 11 illustrates that improved night time control of excess ammonia is achieved with HPN-100.

[00128] Figure 12 shows that especially for patients with higher ammonia levels when treated with sodium PBA (Na PBA), HPN-100 provides better control than sodium PBA, while in patients with lower ammonia levels (ones for whom sodium PBA seems to work relatively well), HPN-100 provides at least comparable ammonia control. Note that for patients having ammonia levels above about 40 μmol/L when treated with sodium PBA, HPN-100 at equimolar dosages provided superior control of ammonia, and consistently reduced ammonia levels to below about 40 μmol/L. Thus for patients whose ammonia levels are abnormal (e.g. above about 40 μmol/L) when treated with sodium PBA, it is

expected that better ammonia control can be achieved with an equimolar amount of HPN-100. Based on this, dosages of HPN-100 can be determined as set forth herein. Figure 13 illustrates that ammonia levels were better controlled in this test by HPN-100 than with sodium PBA, e.g., the average ammonia levels are lower, and tend to be below the upper limit for normal.

Example 4

Relationship Between Ammonia Control and Urinary PAGN Excretion

[00129] As part of the clinical study in UCD patients described in the example above (Example 3), the relationship between plasma ammonia levels and urinary excretion of PAGN was examined. Unlike blood levels of PAA or PBA which exhibited no consistent relationship to ammonia levels (i.e. ammonia control), blood ammonia assessed as the time-normalized area under the curve exhibited an inverse curvilinear relationship to urinary PAGN. That is, plasma ammonia decreased as urinary PAGN increased. Moreover, the relationship between ammonia and urinary PAGN excretion did not differ between sodium PBA and HPN-100 suggesting that this method of dose determination is independent of product formulation. Figure 5 shows a plot of Plasma Ammonia (TN-AUC) versus Urinary PAGN Excretion.

Example 5

Experimentation With Dosing Schedule

[00130] The results of single dose PK/PD modeling observed in the examples above suggested that HPN-100 exhibits delayed release characteristics as compared with sodium PBA with a corresponding potential for increased flexibility in dosing, which was further explored in additional clinical studies described above. In one of these, HPN-100 was administered twice daily as well as in the fasted and fed state. In the other, HPN-100 was administered three times daily with meals. Both 3x daily and 2x daily dosing resulted in a similar proportion of PAGN excreted in the urine and, as demonstrated in adult UCD patients, three times daily dosing was associated with effective ammonia control.

[00131] In Example 2, a number of secondary statistical analyses comparing PK variables after fed versus fasted HPN-100 dosing and single versus multiple HPN-100 dosing were also done. There were no PK or PD differences observed when HPN-100 was administered

after fasting (day 1) or with a meal (day 8). Accordingly, it is believed that HPN-100 can be effectively administered without the need for it to accompany a meal, while the label and package insert for sodium PBA (sodium PBA) indicate that it should be taken with meals. In addition to the lack of difference for PAA PK variables between the fasted and fed states (Days 8 vs 1), the table below also illustrates plasma accumulation of PAA that occurs with multiple dosing (Days 15 vs. 8).

Plasma PK Variables For PAA

PK variable	Child-Pugh A (n = 8)	Child-Pugh B (n = 8)	Child-Pugh C (n = 8)	Healthy volunteers (n = 8)
AUC_{0-12} [(μg/mL)·h]				
Day 1				
Geo. mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00
CV%	53.41	64.91	109.58	79.59
Day 8				
Geo. mean (range)	39.64 (5.96–153.14)	73.44 (26.83–279.48)	86.36 (28.12–367.70)	34.07 (5.27–134.99)
CV%	78.73	85.58	92.85	80.59
Day 15				
Geo. mean (range)	117.89 (23.28–413.43)	138.95 (40.21–652.99)	184.26 (14.97–2245.51)	99.16 (30.06–394.79
CV%	76.82	99.48	170.56	88.59
AUC_{0-t} [(μg/mL)·h]				
Day1				
Geo. Mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00
CV%	53.41	64.91	109.58	79.59
Day 15*				
Geo. Mean (range)	121.57 (23.28–528.73)	153.00 (40.21–938.85)	194.17 (14.97–3415.51)	99.94 (30.06–420.32
CV%	92.27	118.54	198.42	93.08
C_{max} [μg/mL]				
Day 1				
Geo. mean (range)	9.65 (2.58–26.93)	13.52 (6.94–27.97)	10.95 (2.68–40.30)	11.81 (4.14–29.79)
CV%	63.78	57.70	82.65	68.72
Day 8				
Geo. mean (range)	10.21 (1.64–25.66)	14.78 (4.46–42.02)	16.03 (6.49–48.07)	10.03 (2.90–28.43)
CV%	62.25	74.53	72.29	66.97
Day 15†				
Geo. mean (range)	29.07 (7.29–53.48)	25.46 (10.54–65.40)	33.28 (5.03–208.80)	21.92 (7.76–61.31)
CV%	44.21	64.26	121.51	62.88
$t_{1/2}$ [h]‡				
Day 1				
Mean (SD)	0	0	2.10 (0.32)	0
Range			1.88–2.33	
Day 15				
Mean (SD)	1.80 (0.94)	2.76 (1.53)	7.70	1.91 (0.37)
Range	1.01–3.14	1.68–3.84	7.70–7.70	1.68–2.33
T_{max} [h]				
Day 1				
Median (range)	3.50 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (2.00–8.00)	6.00 (4.00–6.00)
Day 8				
Median (range)	4.00 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (4.00–8.00)	4.00 (3.00–6.00)
Day 15				
Median (range)	4.00 (2.00–6.00)	4.00 (3.00–8.00)	5.00 (0.00–8.00)	4.00 (3.00–4.00)

*p = 0.64 for group effect; †p = 0.72 for group effect

‡On day 1, n = 2 in Child-Pugh group B and n = 0 in all other groups; on day 15, n = 4 in group A, 2 in group B, 1 in group C, and 3 in group D

AUC_{0-12}, area under the plasma concentration curve from time 0 up to 12 hours after dosing; AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; geo. Mean, geometric mean; n, number of subjects; SD, standard deviation; T_{max}, time to maximum observed plasma concentration; $t_{1/2}$, half-life

Example 6

PK/PD Modeling Results

[00132] In the case of most drugs, the fraction of an orally administered dose which is removed and metabolized by the liver prior to reaching the systemic circulation (i.e. first pass effect) is not considered bioavailable, since it does not enter the systemic circulation and therefore is not able to reach its target organ or receptor. However, this is not the case for ammonia scavenging drugs described in this invention. Since hepatocytes and possibly enterocytes contain the enzymes necessary for conversion of PBA to PAA and conversion of PAA to PAGN and since glutamine is present in the splanchnic as well as the systemic circulation, it is likely that PBA can be converted to PAGN prior to reaching the systemic circulation (i.e. "pre-systemically") and that this PBA is fully effective with respect to ammonia scavenging (Figure 6); i.e. fully active. To verify this possibility, PK/PD modeling using NONMEM VI (Icon, Ellicot City, MD.) was carried out on plasma and urinary metabolite data (over 5000 data points) from the clinical studies described above involving healthy adults, subjects with cirrhosis and UCD subjects. The results of this PK/PD modeling have validated the model depicted in Figure 3. Moreover, the modeling has verified that HPN-100 exhibits slow release characteristics as compared with sodium PBA and provided an explanation for the poor correlation between blood levels of PBA/PAA and ammonia and the importance of urinary PAGN is dose adjustment. Key conclusions resulting from the PK/PD modeling were as follows

1. PBA is more slowly absorbed (~40% as fast) from the intestine after administration of HPN-100 versus sodium PBA (absorption rate constants and absorption half-lives for HPN-100 and sodium PBA are 0.544 h^{-1} vs. 1.34 h^{-1} and 1.27 h vs. 0.52 h, respectively).

2. The lower plasma levels of PBA following administration of HPN-100, as compared with sodium PBA, reflect results indicating a fractionally greater amount of PBA (31% vs. 1%) being converted pre-systemically (to PAA and PAGN) following administration of HPN-100 than Na PBA.

3. In a dataset containing healthy, cirrhotic, and UCD individuals, diagnosis was introduced as a covariate on the estimated bioavailability of HPN-100 revealing a 32% lower estimated bioavailability of PBA in healthy adults compared to adult UCD patients. Cirrhotic and UCD patients had similar PBA bioavailability following HPN-100 treatment.

Example 7

ADME Study In Three Cynomolgous Monkeys

[00133] To assess 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. This study corroborated clinical findings summarized in Examples 1-3, including the following: (a) dosing with oral sodium PBA or oral HPN-100 did not result in 100% conversion to urinary PAGN, (b) plasma PBA and PAA blood levels did not correlate consistently with ammonia scavenging activity as reflected by urinary PAGN output, and (c) HPN-100 exhibited slow release characteristics as compared with sodium PBA.

[00134] Radio labeled PBA and PAA entered the systemic circulation rather slowly following administration of radio labeled HPN-100 [Cmax for PBA was achieved 1.5 hours post-dosing (52.2 μg/mL) and Cmax for PAA was achieved 8 hours post dosing (114 μg/mL)], corroborating the findings observed in humans (including the PK/PD modeling), and essentially no HPN-100 appeared in systemic circulation or in excretions. About 90% of radioactive material derived from HPN-100 that was excreted in urine was PAGN, accounting for 39% of the administered HPN-100. By contrast, when oral sodium PBA was administered, PAGN accounted for only 23% of the radio labeled material, and unchanged PBA accounted for 48% of the administered dosage of oral sodium PBA. Thus oral sodium PBA was utilized less efficiently than HPN-100, and an unexpectedly high amount of PBA was excreted unchanged.

Example 8

Biological and Anatomical Considerations

[00135] Unlike most drugs which act on a target organ/cell/receptor (etc.) perfused by systemic blood, ammonia scavenging drugs of the types covered by this invention do not act on a target organ, rather they act through the combination of PAA with glutamine to form PAGN (Figure 6). Since glutamine is present in the splanchnic as well as the systemic circulation and since the liver is a metabolically active organ capable of catalyzing all steps involved in the conversion of HPN-100 or PBA to PAA and then to PAGN, the data accumulated to date, including the PK/PD modeling, as well as anatomical consideration

lead us to the conclusion that the formation of PAGN from PBA/PAA occurs to a significant degree before PBA/PAA reach the systemic circulation (e.g. within the liver). This is especially true when HPN-100 is administered as a PBA prodrug. This explains the poor correlation between plasma levels and ammonia trapping effects and leads to the conclusion that the dosing and dose adjustment of these PBA prodrugs should be based on urinary excretion of PAGN and total urinary nitrogen. Figure 6 illustrates how this occurs.

[00136] For certain clinical trials, particularly for comparing HPN-100 to PBA, HPN-100 will be administered at a dose that is equivalent (equimolar) to an amount of sodium PBA that would be considered suitable for the particular patient; and the dosage can then be adjusted by the methods described herein. For example, the HPN-100 dose range will match the PBA molar equivalent of the approved sodium PBA (sodium phenylbutyrate) (NaPBA) dose range. HPN-100 will be administered three times a day (TID) with meals. Note that the conversion of the dose of NaPBA to the dose of HPN-100 involves correction for their different chemical forms (i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium) (NaPBA [g] x 0.95 = HPN-100 [g]) as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

HPN-100 Dose Ranges Corresponding to Recommended Daily Doses of Sodium PBA

Sodium PBA	HPN-100 PBA Equivalent Dose (mg)	HPN-100 PBA Equivalent Dose (mL)
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[1] 20 g of sodium PBA contains ~17.6 g of phenylbutyric acid; 19 g of HPN-100 contains ~17.6 g of phenylbutyric acid

Example 9

Determination of a Starting Dosage and Dose Adjustment of HPN-100

[00137] A patient having a nitrogen retention state (e.g. an inherited urea cycle disorder or cirrhosis) who is currently not being treated with an ammonia scavenging agent as described in this invention is determined clinically to be in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of HE in patients with cirrhosis, elevated blood ammonia levels).