IN THE UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF TEXAS
WACO DIVISION

ZANOPRIMA LIFESCIENCES, LTD, Plaintiff, v. HANGSEN INTERNATIONAL GROUP LTD, Defendant.	§ § § § § § § § § §	Civil Action No. 22-268

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiff Zanoprima Lifesciences, Ltd. ("Zanoprima" or "Plaintiff"), by and through its undersigned counsel, pleads the following against Defendant Hangsen International Group Ltd. ("Hangsen" or "Defendant"), and alleges as follows:

THE PARTIES

1. Plaintiff Zanoprima is a corporation duly organized and existing under the laws of the United Kingdom. Zanoprima's principal place of business is 5th Floor, Charles House, 108-110 Finchley Road, London NW3 5JJ, Great Britain.

2. On information and belief, Hangsen is a corporation duly organized and existing under the laws of China. Further, on information and belief, Hangsen maintains a regular and established place of business in Shenzhen, Guangdong, China and/or TsimSha Tsui, Kowloon, Hong Kong.

BACKGROUND, JURISDICTION, AND VENUE

3. This is an action arising under the patent laws of the United States, 35 U.S.C. § 1 *et seq*. Accordingly, this Court has subject matter jurisdiction pursuant to 28 U.S.C. §§ 1331 and 1338(a).

A. Zanoprima

4. Zanoprima was founded in June 2014 by Ashok Narasimhan and Nicholas Hyde with the aim of reducing tobacco-related harm by designing and developing replacement products. Sadly, Nicholas Hyde passed away in September 2020 following a battle with lung cancer.

5. The vision for Zanoprima arose from a concern in the pharmaceutical industry regarding the provenance and purity of tobacco-derived nicotine being used in the very products intended to reduce addiction to tobacco as well as disease and death associated with tobacco. In response to this concern, Zanoprima sought to develop a process of nicotine synthesis that would create nicotine chemically identical to that derived from the tobacco plant but (a) was devoid of tobacco specific nitrosamines, carcinogens, tobacco alkaloids, and other impurities, (b) was traceable with high levels of enantiomeric and chemical purity, and (c) could rival the pricing of tobacco-derived nicotine. Unfortunately, at that time, there was no economically-viable method to synthesize and bulk-produce (S)-nicotine, the predominant and more active enantiomer of nicotine found in the tobacco plant.

6. In November 2014, Zanoprima's researchers began research and development of a synthesis approach that involved an intermediate compound that had been largely overlooked in nicotine research, (S)-nornicotine. Zanoprima's researchers ultimately envisioned a process whereby biocatalysis, using an enzyme, could produce an enantiomerically pure (S)-nornicotine, which could then be methylated to produce the desired enantiomerically pure (S)-nicotine. However, transitioning this concept to reality required a significant investment in time and resources. Zanoprima worked tirelessly to screen and identify which enzymes to use, to improve the enzyme reaction, and to optimize the methylation of (S)-nornicotine.

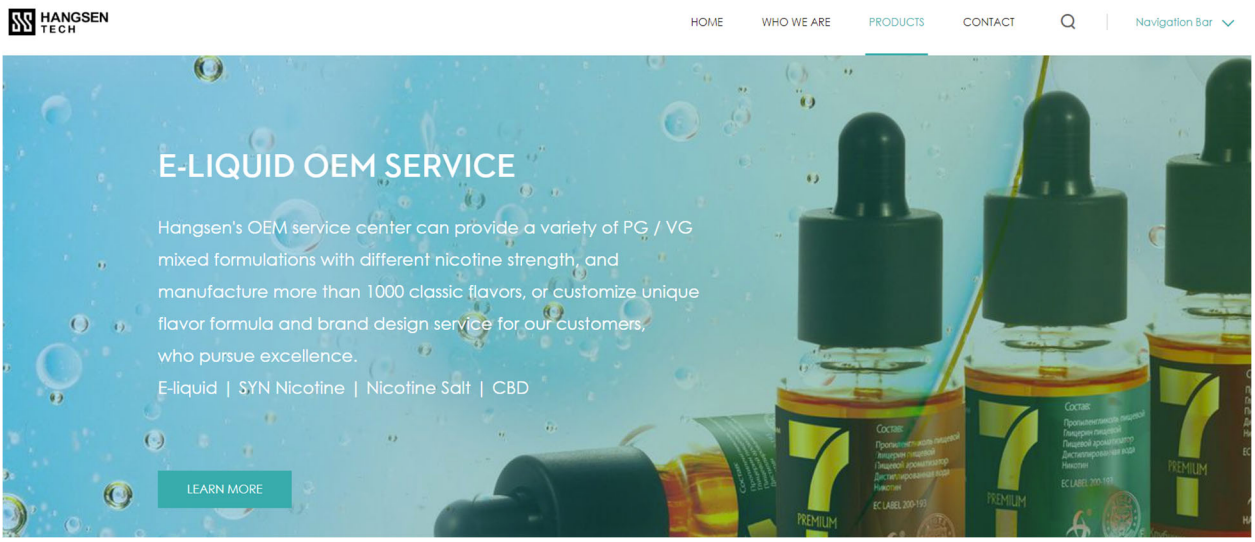
7. After nearly four years of extensive research and substantial investment, by November 2018, Zanoprima had developed a novel and reliable method to synthesize (S)-nicotine

and promptly filed that patent application on the method that would ultimately issue as U.S. Patent No. 10,913,962 (the "'962 Patent").

8. Zanoprima manufactures synthetic (S)-nicotine using its patented method in an FDA-approved manufacturing facility.[1] Zanoprima markets and sells its synthetic (S)-nicotine, including in the United States, with the goal of facilitating the movement towards a tobacco-free world.[2]

B. Hangsen

9. Hangsen is a manufacturer and distributor of e-cigarettes, e-liquid, nicotine salt, and synthetic nicotine.[3]



10. Hangsen directly competes with Zanoprima in the marketplace. For example, Hangsen imports and sells synthetic (S)-nicotine and products that include synthetic (S)-nicotine (the "Accused Products") in the United States. The Accused Products directly compete with Zanoprima's patented synthetic (S)-nicotine products.

[1] https://www.zanoprima.com/our-products
[2] https://www.zanoprima.com/
[3] https://www.hkhangsen.com/list/index/cid/7.html

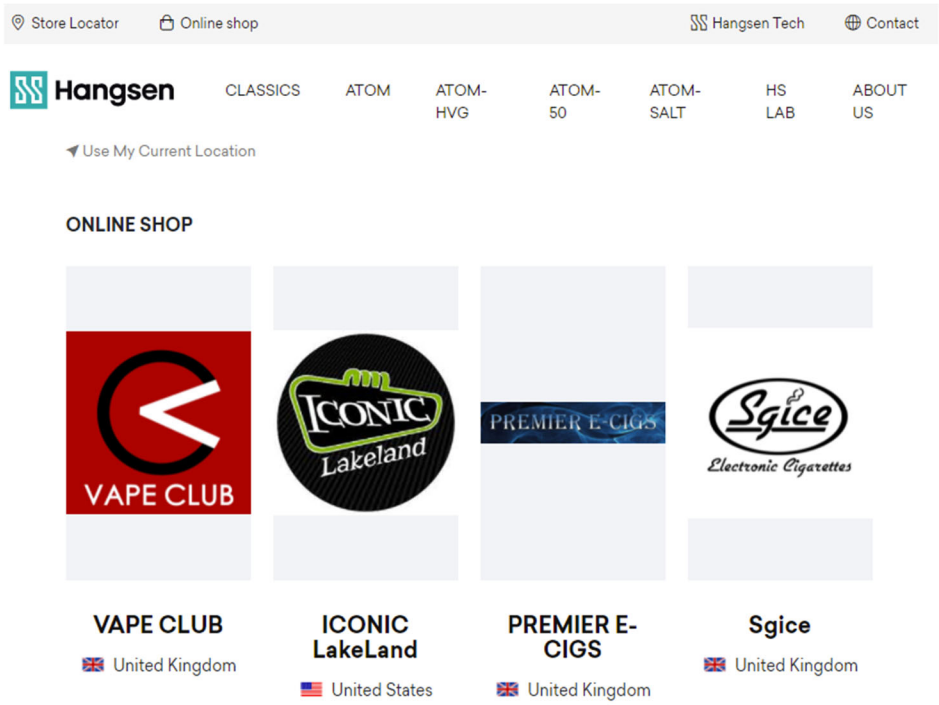
11. Hangsen infringes the ’962 Patent by selling, offering for sale, using, making, and/or importing the Accused Products into the United States.

12. Hangsen is subject to this Court’s personal jurisdiction because Hangsen has sufficient minimum contacts within Texas pursuant to due process and the Texas Long Arm Statute, Tex. Civ. Prac. & Rem. Code § 17.042.

13. Hangsen has purposefully directed its products, including the Accused Products, and activities at the United States, including Texas, and has engaged in conduct that indicates an intent or purpose to serve the market in the United States and Texas, such as designing its products broadly for the United States market, advertising in the United States, and marketing and selling its products through one or more distributors in the United States and Texas.

14. Hangsen has sold or distributed products to multiple U.S. distributors, who sell their products throughout the United States, including in Texas. On information and belief, Hangsen has sold the Accused Products to Triton Distribution Group, a distributor located in Richardson, Texas. Furthermore, on information and belief, Hangsen directs online shoppers to purchase Hangsen products from U.S. distributors, which can be purchased from those U.S. distributors and shipped throughout the United States, including Texas.[4]

[4] https://www.hangsen.com/online-shop

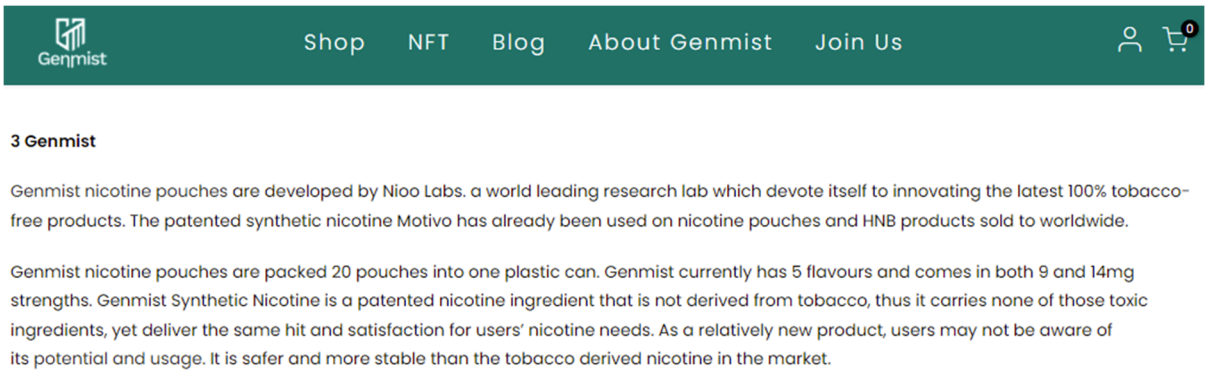


15. Hangsen has distributed its products, including the Accused Products, to retailers and manufacturers throughout the United States, including Texas. For example, upon information and belief, Vapetasia, who has a principal place of business is Richardson, Texas, is a manufacturer of final consumer products, including Vapetasia Salts E-Liquid, that incorporate the Accused Products. Vapetasia Salts E-Liquid is available for sale within the state of Texas. On March 8, 2022, four packages of Vapetasia Salts E-Liquid were purchased at Austin Pineapple Express, a retailer at 3401 Guadalupe Street in Austin, Texas 78705. A true and correct copy of a picture of the receipt and the four packages is attached as Exhibit 1 and incorporated herein.

16. Additionally, as another example, on September 10, 2020, when Hangsen "announced the global release of SYN Nicotine," Hangsen also stated that "SYN Nicotine e-liquids will be used in Geek Bar's product, which is set to launch soon in the North America

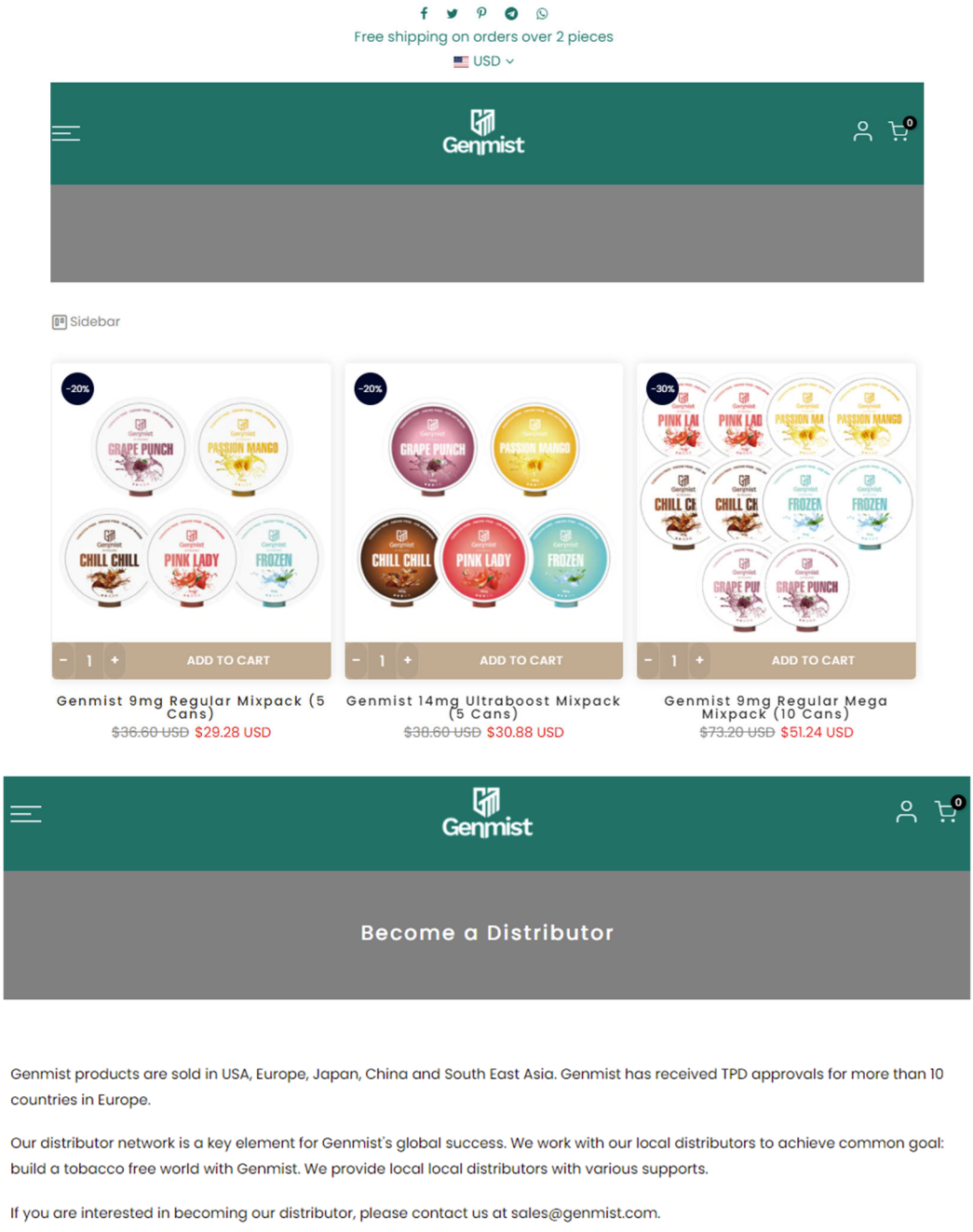
market."[5] Indeed, Geek Bar currently advertises on its "Synthetic Nicotine Disposables" webpage that "[t]he inaugural batch of custom-made SYN Nicotine e-liquids will be used in Geek Bar products. And in the foreseeable future we will continue to use tobacco-free nicotine in all our existing and up-coming products …."[6] On information and belief, Geek Bar products are sold in Texas through Texas distributors and include the Accused Products.

17. On information and belief, Hangsen also provides, sells, and/or offers to sell Accused Products to Genmist, a company that sells nicotine pouches throughout the world, including the United States. Genmist advertises on its website that Genmist nicotine pouches utilize "[t]he patented synthetic nicotine Motivo [that] has already been used on nicotine pouches and HNB products sold to worldwide."[7]



18. On information and belief, Genmist has physical locations within the United States and makes, sells, or offers to sell its nicotine pouches that include the Accused Products to customers within the United States, including Texas:[8]

[5] https://apnews.com/press-release/pr-newswire/corporate-news-products-and-services-tobacco-products-manufacturing-technology-consumer-products-and-services-625259e1af8f52e68920659dbfa60f58
[6] http://www.geekbar.com/article/detail.html?id=8
[7] https://www.genmist.shop/blogs/genmist-blog/top-nicotine-pouch-brands
[8] https://www.genmist.shop/collections/nicotine-pouches; https://www.genmist.shop/pages/distributorship



19. Upon information and belief, Hangsen both operates a U.S.-based center that engages in the importation of Hangsen products into the United States as well as the distribution and/or sale of Hangsen products throughout the United States, including Texas, and actively

promotes itself and makes public its purposeful availment of the United States', including Texas' market.[9]

20. Venue is proper in this judicial District pursuant to at least 28 U.S.C. §§ 1391(b), 1391(c), and 1400(b), as well as under the "alien venue rule." *Brunette Machine Works, Ltd. v. Kockum Indus., Inc.*, 406 U.S. 706 (1972); *In re HTC Corp.*, 889 F.3d 1349 (Fed. Cir. 2018). As noted above, Hangsen is a foreign entity that is subject to personal jurisdiction in this Court.

THE ASSERTED PATENT AND THE ACCUSED PRODUCTS

C. The Asserted Patent

21. On February 9, 2021, the '962 Patent, entitled "Process of Making (S)-Nicotine," was duly and legally issued by the United States Patent and Trademark Office. A true and correct copy of the '962 Patent is attached as Exhibit 2 and incorporated herein.

22. The '962 Patent names Raymond McCague and Ashok Srinivasan Narasimhan as co-inventors.

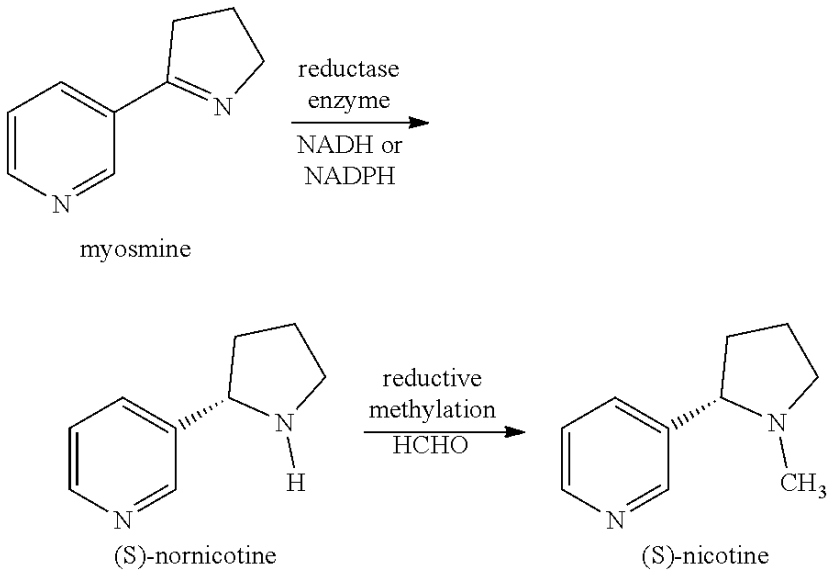
[9] https://www.hkhangsen.com/list/index/cid/3.html

23. The ’962 Patent claims priority to European Patent Office Application No. 18206826, which has a filing date of November 16, 2018.

24. The ’962 Patent has been in full force and effect since its issuance. Zanoprima is the owner, by assignment, of all rights, title, and interest in and to the ’962 Patent, including the right to seek damages for past, current, and future infringement thereof.

25. The ’962 Patent states that it “relates to a process for synthetically producing (S)-nicotine ([(S)-3-(1-methylpyrrolidin-2-yl) pyridine]).” Ex. 2 at 1:5–7.

26. Claim 1 of the ’962 Patent recites: “A process of making (S)-nicotine comprising the steps of: (i) reducing myosmine with an enzyme with imine reductase activity to form (S)-nornicotine; and (ii) methylating the (S)-nornicotine formed from step (i) to form (S)-nicotine; wherein step (ii) is carried out by way of reductive methylations; and wherein in step (ii) the (S)-nornicotine is reductively methylated, using formaldehyde or a formaldehyde-based compound in the presence of a reductant.” Ex. 2 at 19:59–20:59. The ’962 Patent discloses the following reaction scheme:



Ex. 2 at 7:41–59.

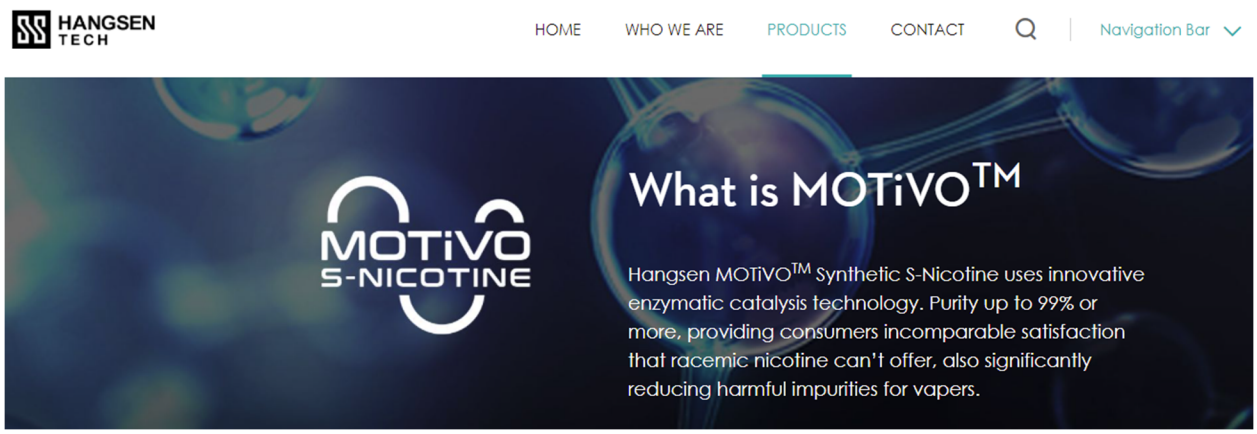
D. The Accused Products

27. Hangsen's Accused Products that infringe the '962 Patent include Hangsen's alleged high-purity synthetic (S)-nicotine and nicotine products that are marketed and sold under various names including as MOTiVO Synthetic S-Nicotine ("MOTiVO"). For example, one United States retailer has advertised its "Island Breeze" product as containing "Motivo S-Nicotine" using Hangen's MOTiVO logo and referring to the product's "Tobacco Free Nicotine:"

https://www.ecigcharleston.com/Island-Breeze-Synthetic-60ml-p/islandbreezesyn60.htm.

28. According to Hangsen's website, Hangsen's "MOTiVO Synthetic S-Nicotine uses innovative enzymatic catalysis technology" with "[p]urity up to 99% or more," which "provid[es] consumers incomparable satisfaction that racemic nicotine can't offer."[10]

[10] https://www.hkhangsen.com/show/index/cid/7/id/6.html



29. On November 18, 2020—after the publication of Zanoprima's United States patent application for the '962 Patent—Hangsen filed Chinese Patent Application No. 112409327A (the "'327 Application"). The '327 Application claims a process that utilizes imine reductase enzymes to convert myosmine into (S)-nornicotine, which then undergoes a reductive methylation reaction with formaldehyde or formaldehyde-based compounds to create (S)-nicotine. Upon information and belief, on or about June 17, 2021, the Chinese Patent Office rejected the '327 Application because it lacked novelty over the PCT application for Zanoprima's '962 Patent.

30. On information and belief, Hangsen manufactures the Accused Products, such as MOTiVO, in accordance with the methods described in the rejected '327 Application in a manner that infringes the '962 Patent.

31. On information and belief, Hangsen also provides, sells, and/or offers to sell MOTiVO-branded Accused Products to Genmist, a company that sells nicotine pouches throughout the world, including the United States. Genmist advertises on its website that Genmist nicotine pouches utilize "[t]he patented synthetic nicotine Motivo [that] has already been used on nicotine pouches and HNB products sold to worldwide."[11]

[11] https://www.genmist.shop/blogs/genmist-blog/top-nicotine-pouch-brands

E. Hangsen's Knowledge of the Asserted Patents and Accused Products

32. On December 20, 2021, counsel for Zanoprima sent a letter to Hangsen directors and legal counsel providing notice of the Asserted Patent ("Notice Letter"). Attached as Exhibit 3 is a true and correct copy of the Notice Letter, which is incorporated herein.

33. In the Notice Letter, Zanoprima notified Hangsen that the Accused Products infringe the '962 Patent.

34. Moreover, on information and belief, based on the rejection for the '327 Application that Hangsen received from the Chinese Patent Office on or about June 17, 2021, Hangsen was aware that the method described in the '327 Application was already described in the '962 Patent, and that any product produced by such method and imported, sold, or used in the United States would be infringing Zanoprima's '962 patent.

CLAIM

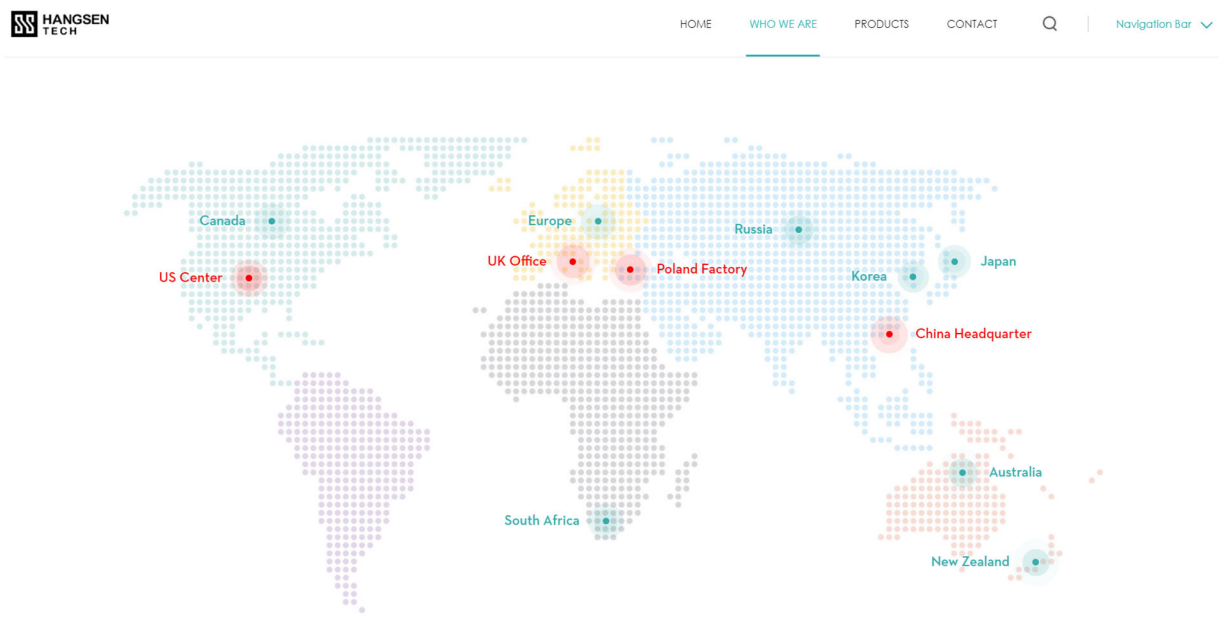
(Infringement of U.S. Patent No. 10,913,962)

35. Zanoprima re-alleges and incorporates herein by reference all previous factual allegations.

36. On information and belief, Hangsen has directly infringed and continues to infringe at least claim 1 of the '962 patent literally or under the doctrine of equivalents by importing into the United States, using, selling, and/or offering for sale in the United States, without authority or license, the Accused Products, in violation of at least 35 U.S.C. § 271(g).

37. On information and belief, Hangsen imports large quantities of the Accused Products for sale and distribution to customers located in the United States. In addition, Hangsen publicly admits that it operates a U.S.-based center that engages in the importation of Accused

Products into the United States as well as the distribution and/or sale of the Accused Products in the United States.[12]



38. On information and belief, Hangsen sells and/or offers for sale the Accused Products within the United States. On information and belief, employees of Hangsen knowingly engage in email correspondence with U.S. manufacturers, distributors, and customers to offer to sell and sell the Accused Products. On information and belief, this email correspondence includes specific prices per amount of Accused Product to be imported into the United States.

39. On information and belief, the Accused Products are manufactured by a process that practices every step of at least claim 1 of the '962 Patent. For example, upon information and belief, during the manufacture of the Accused Products, myosmine is reduced with an imine reductase enzyme to form (S)-nornicotine. Further, upon information and belief, the (S)-nornicotine is reductively methylated to (S)-nicotine using formaldehyde or a formaldehyde-based compound in the presence of a reductant. Upon information and belief, use of a formaldehyde or

[12] https://www.hkhangsen.com/list/index/cid/3.html

a formaldehyde-based compound to reductively methylate the (S)-nornicotine is the only commercially-viable method to perform that step.

40. On information and belief, Hangsen's manufacture of the Accused Products utilizes an imine reductase enzyme to reduce myosmine to (S)-nornicotine. On information and belief, Hangsen utilizes an imine reductase enzyme to reduce myosmine to (S)-nornicotine in accordance with the processes described in its rejected '327 Application.

41. On information and belief, Hangsen's manufacture of the Accused Products uses formaldehyde or a formaldehyde-based compound in the presence of a reductant.

42. On information and belief, the Accused Products are neither materially changed by subsequent process nor become trivial and nonessential components of another product. To the contrary, the synthetic (S)-nicotine of the Accused Products is an essential component of final commercial products.

43. On information and belief, Hangsen has knowingly induced and continues to induce others, including its affiliates, suppliers, contract manufacturers, distributors, and customers to infringe one or more claims of the '962 Patent, including, but not limited to, claim 1, pursuant to 35 U.S.C. § 271(b), by actively encouraging and/or instructing others to import into the United States or make, use, sell, and/or offer to sell in the United States the Accused Products.

44. On information and belief, Hangsen sells or offers for sell the Accused Products to third parties that incorporate the Accused Products into third party products (the "Third Party Products"). On information and belief, Hangsen assists third parties, directly and/or through intermediaries, in the development of the Third Party Products and supports the sales of the Third Party Products.

45. On information and belief, Hangsen has had the specific intent, or has been willfully blind, to induce others to infringe the '962 Patent including inducing others to import, make, use, sell, and/or offer to sell, in the United States, the Third Party Products that include Accused Products, whose manufacture, use, sale, offer for sale, or importation in the United States constitute direct infringement of at least one claim of the '962 Patent. On information and belief, since at least the date of the Notice Letter, Hangsen has known that third parties who import, sell, offer to sell, or use the Accused Products in the United States are directly infringing the '962 Patent.

46. On information and belief, the Third Party Products are imported into the United States, or manufactured in the United States, for use, sale, and/or offer for sale in Texas and throughout the United States.

47. On information and belief, to the extent any entity other than Hangsen, including but not limited to any of Hangsen's partners, subsidiaries, parent companies, or agents, imports the Accused Products or the Third Party Products into the United States for or on behalf of Hangsen (the "Third Party Importer"), Hangsen is liable for inducement of infringement by the Third Party Importer. Hangsen has encouraged the Third Party Importer to infringe the '962 Patent and intended that it do so. This encouragement includes assisting, ordering or instructing the Third Party Importer to import the Accused Products and/or Third Party Products into the United States, providing directions and other materials to the Third Party Importer to enable such importation, and/or conditioning the receipt of benefits (including but not limited to payment) to the Third Party Importer on such importation. On information and belief, this behavior has continued at least since Hangsen first became aware of the '962 Patent and the infringement thereof.

48. On information and belief, Hangsen imports into the United States or sells or offers to sell within the United States materials and apparatuses that contribute to the direct infringement

of others within the United States, including its affiliates, suppliers, contract manufacturers, distributors, and customers. For example, the Accused Products constitute a material part of one or more claims of the '962 Patent and are not staple articles or commodities of commerce suitable for substantial noninfringing use. On information and belief, since at least the date of the Notice Letter, Hangsen has known that the Accused Products constitute a material part of the inventions of the '962 Patent and are not staple articles or commodities of commerce suitable for substantial noninfringing use. On information and belief, Hangsen sells or offers to sell the Accused Products to third parties, such as affiliates, suppliers, contract manufacturers, distributors, and customers, who directly in infringe the '962 Patent by selling, offering for sell, using, making and/or importing without authority or license, Third Party Products that include the Accused Products.

49. Hangsen has benefitted and continues to benefit from the importation into the United States and the use, sale, or offer of sale in the United States of the Accused Products or the Third Party Products.

50. Zanoprima has suffered, and continues to suffer, damages as a result of Hangsen's infringement of the '962 Patent.

51. Hangsen has continued to infringe the '962 Patent since at least December 20, 2021, despite being on notice of the '962 Patent and its infringement. Hangsen has therefore infringed the '962 Patent knowingly, willfully, deliberately, and in disregard of Zanoprima's patent rights since at least December 20, 2021, at least by performing acts of infringement with actual knowledge of its direct and indirect infringement or while remaining willfully blind to the fact of its direct and indirect infringement. As a result of at least this conduct, Zanoprima is entitled to enhanced damages under 35 U.S.C. § 284 and to attorneys' fees and costs under 35 U.S.C. § 285.

52. Zanoprima reserves the right to modify its infringement theories as discovery progresses in this case. Zanoprima shall not be estopped or otherwise limited or restricted for the purposes of its infringement contentions or its claim constructions by allegations provided within this Complaint.

SUPPORT FOR PRELIMINARY AND PERMANENT INJUNCTION

53. Zanoprima incorporates by reference all previous factual allegations.

54. Immediate and irreparable harm has resulted, and will continue to result, from Hangsen's infringement of the '962 Patent.

55. Because Zanoprima's remedy at law is inadequate, Zanoprima seeks preliminary and permanent injunctive relief. Zanoprima is threatened with losing market share and competitive advantage, in amounts that may be impossible to determine, unless Hangsen is enjoined and restrained by Order of the Court.

56. The hardship of Hangsen's continued infringement of the '962 patent on Zanoprima, including the hardship of not being able to exclude others from practicing its patented invention, outweighs any potential hardship on Hangsen. As such, a remedy in equity is warranted.

57. Public policy supports granting preliminary and permanent injunctive relief to prevent further infringement of the '962 Patent.

PRAYER FOR RELIEF

WHEREFORE, Zanoprima prays for judgment against Defendant as follows:

A. The Defendant has infringed and, unless enjoined, will continue to infringe the '962 Patent;

B. The Defendant has willfully infringed the '962 Patent;

C. That Defendant be enjoined from infringing the ’962 Patent, or if its infringement is not enjoined, that Defendant be ordered to pay ongoing royalties to Zanoprima for any post-judgment infringement of the ’962 Patent;

D. The Defendant pay Zanoprima damages to compensate Zanoprima for Defendant’s past infringement and any continuing or future infringement of the ’962 Patent, together with interest and costs, under 35 U.S.C. § 284;

E. That Defendant be ordered to pay prejudgment and post-judgment interest on the damages assessed;

F. That Defendant pay Zanoprima enhanced damages pursuant to 35 U.S.C. § 284;

G. That Defendant be ordered to pay supplemental damages to Zanoprima, including interest, with an accounting, as needed;

H. That this is an exceptional case under 35 U.S.C. § 285, and that Defendants pay Zanoprima’s attorneys’ fees and costs in this action; and

I. That Zanoprima be awarded such other and further relief, including equitable relief, as this Court deems just and proper.

DEMAND FOR JURY TRIAL

Pursuant to Federal Rule of Civil Procedure 38(b), Zanoprima hereby demands a trial by jury on all issues triable to a jury.

Dated: March 12, 2022

Respectfully submitted,

/s/ Marc B. Collier

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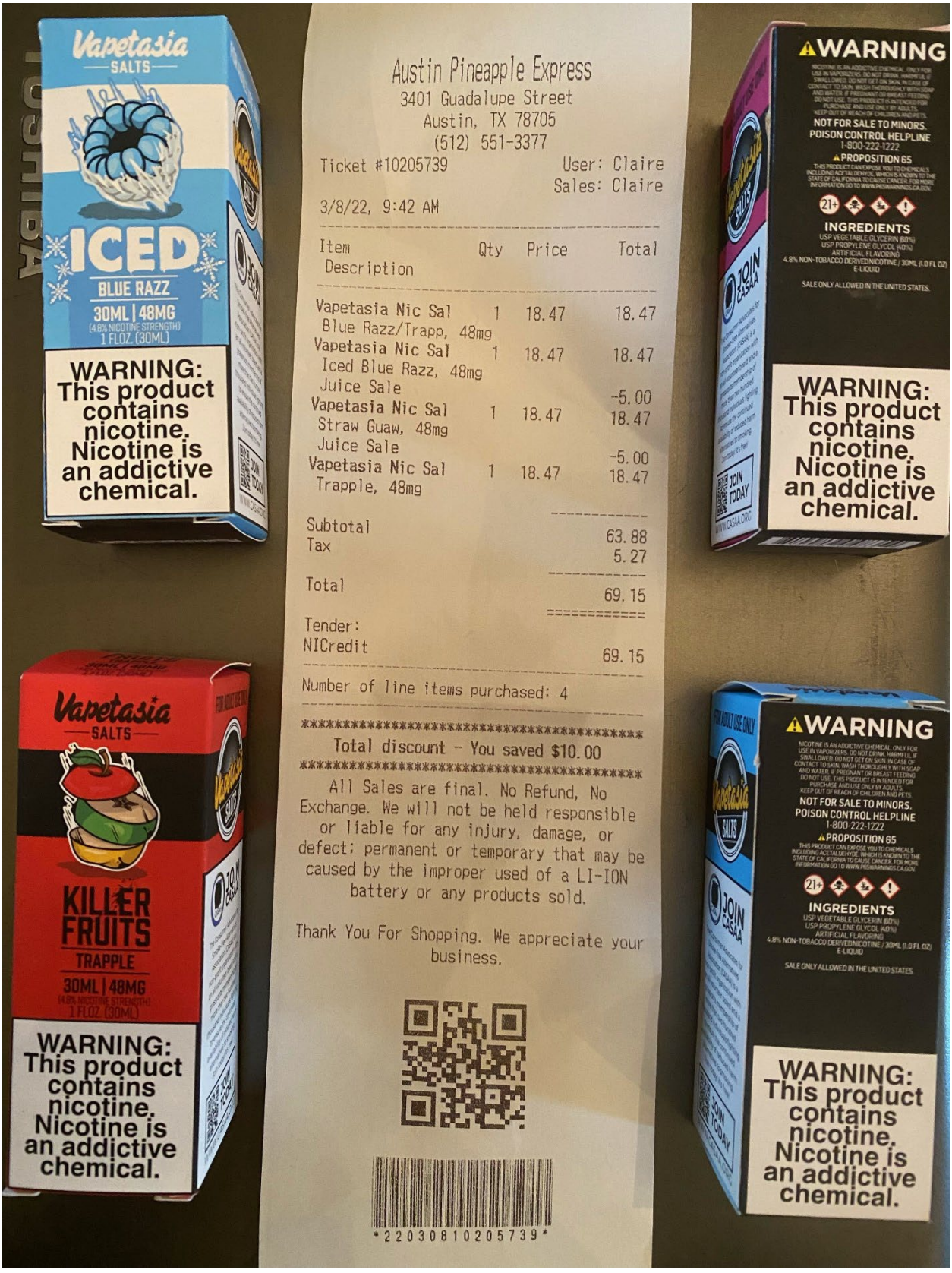
Counsel for Plaintiff Zanoprima Lifesciences Ltd.

EXHIBIT 1


Vapetasia
SALTS
ICED
BLUE RAZZ
30ML | 48MG
(4.8% NICOTINE STRENGTH)
1 FLOZ (30ML)
WARNING: This product contains nicotine. Nicotine is an addictive chemical.
Austin Pineapple Express
3401 Guadalupe Street
Austin, TX 78705
(512) 551-3377
Ticket #10205739
User: Claire
Sales: Claire
3/8/22, 9:42 AM
Item Description Qty Price Total
Vapetasia Nic Sal Blue Razz/Trapp, 48mg 1 18.47 18.47
Vapetasia Nic Sal Iced Blue Razz, 48mg 1 18.47 18.47
Juice Sale -5.00
Vapetasia Nic Sal Straw Guaw, 48mg 1 18.47 18.47
Juice Sale -5.00
Vapetasia Nic Sal Trapple, 48mg 1 18.47 18.47
Subtotal 63.88
Tax 5.27
Total 69.15
Tender:
NICredit 69.15
Number of line items purchased: 4
Total discount - You saved $10.00
All Sales are final. No Refund, No Exchange. We will not be held responsible or liable for any injury, damage, or defect; permanent or temporary that may be caused by the improper used of a LI-ION battery or any products sold.
Thank You For Shopping. We appreciate your business.
220308102057 39
WARNING
NOT FOR SALE TO MINORS.
POISON CONTROL HELPLINE
1-800-222-1222
PROPOSITION 65
INGREDIENTS
USP VEGETABLE GLYCERIN (60%)
USP PROPYLENE GLYCOL (40%)
ARTIFICIAL FLAVORING
4.8% NON-TOBACCO DERIVED NICOTINE / 30ML (1.0 FL OZ)
E-LIQUID
SALE ONLY ALLOWED IN THE UNITED STATES
JOIN CASAA
JOIN TODAY
WWW.CASAA.ORG
WARNING: This product contains nicotine. Nicotine is an addictive chemical.
Vapetasia
SALTS
KILLER FRUITS
TRAPPLE
30ML | 48MG
1 FLOZ (30ML)
WARNING: This product contains nicotine. Nicotine is an addictive chemical.
FOR ADULT USE ONLY
WARNING
NOT FOR SALE TO MINORS.
POISON CONTROL HELPLINE
1-800-222-1222
PROPOSITION 65
INGREDIENTS
USP VEGETABLE GLYCERIN (60%)
USP PROPYLENE GLYCOL (40%)
ARTIFICIAL FLAVORING
4.8% NON-TOBACCO DERIVED NICOTINE / 30ML (1.0 FL OZ)
E-LIQUID
SALE ONLY ALLOWED IN THE UNITED STATES
JOIN CASAA
JOIN TODAY
WARNING: This product contains nicotine. Nicotine is an addictive chemical.

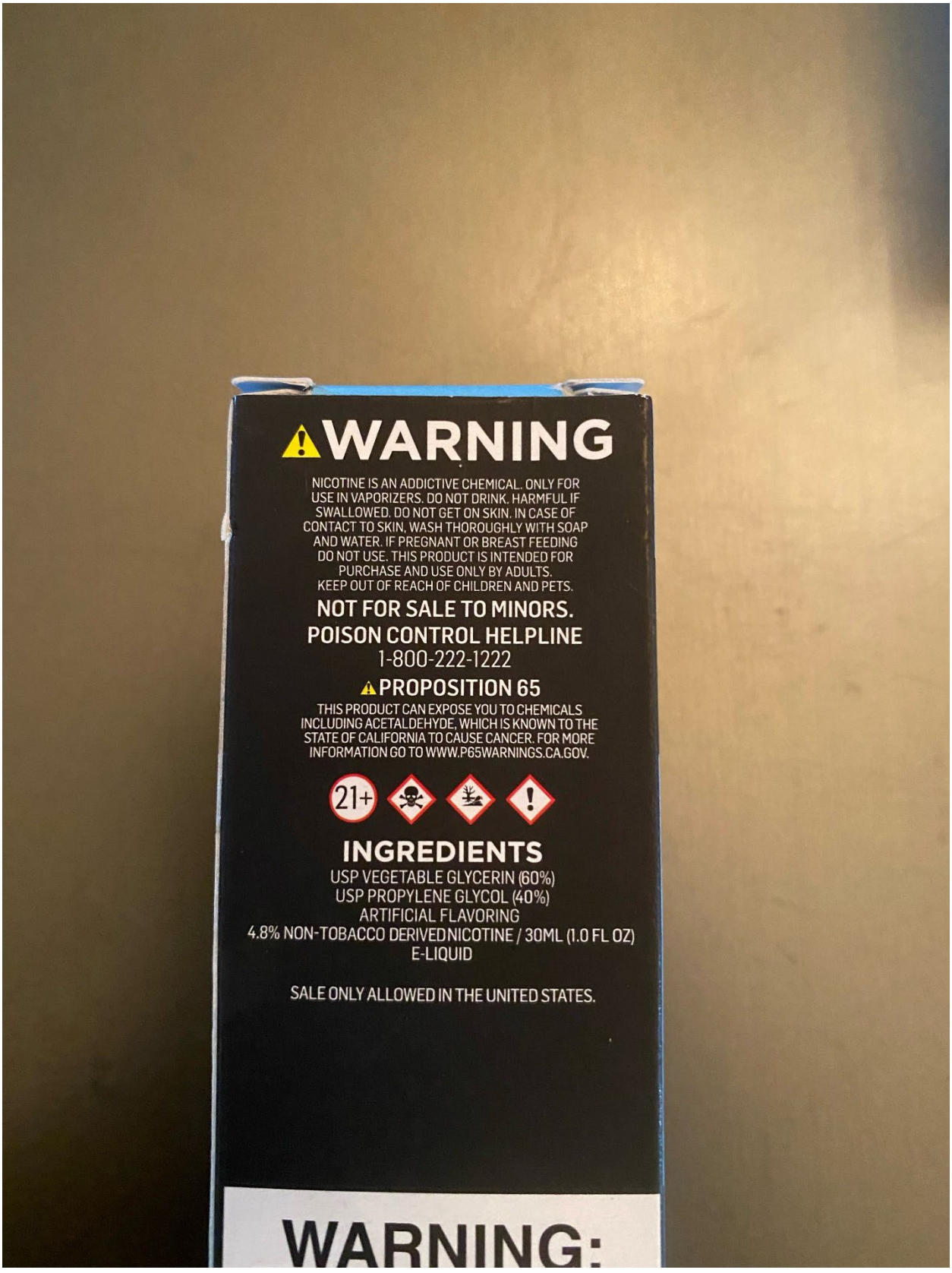

WARNING
NICOTINE IS AN ADDICTIVE CHEMICAL. ONLY FOR USE IN VAPORIZERS. DO NOT DRINK. HARMFUL IF SWALLOWED. DO NOT GET ON SKIN. IN CASE OF CONTACT TO SKIN, WASH THOROUGHLY WITH SOAP AND WATER. IF PREGNANT OR BREAST FEEDING DO NOT USE. THIS PRODUCT IS INTENDED FOR PURCHASE AND USE ONLY BY ADULTS. KEEP OUT OF REACH OF CHILDREN AND PETS.
NOT FOR SALE TO MINORS.
POISON CONTROL HELPLINE
1-800-222-1222
PROPOSITION 65
THIS PRODUCT CAN EXPOSE YOU TO CHEMICALS INCLUDING ACETALDEHYDE, WHICH IS KNOWN TO THE STATE OF CALIFORNIA TO CAUSE CANCER. FOR MORE INFORMATION GO TO WWW.P65WARNINGS.CA.GOV.
21+
INGREDIENTS
USP VEGETABLE GLYCERIN (60%)
USP PROPYLENE GLYCOL (40%)
ARTIFICIAL FLAVORING
4.8% NON-TOBACCO DERIVED NICOTINE / 30ML (1.0 FL OZ)
E-LIQUID
SALE ONLY ALLOWED IN THE UNITED STATES.
WARNING:

EXHIBIT 2

US010913962B2

(12) **United States Patent**
McCague et al.

(10) **Patent No.: US 10,913,962 B2**
(45) **Date of Patent: Feb. 9, 2021**

(54) **PROCESS OF MAKING (S)-NICOTINE**

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CPC ***C12P 17/165*** (2013.01); *C12Y 105/01* (2013.01)

(58) **Field of Classification Search**
CPC C07D 401/04; C12P 17/165; C12Y 105/01
See application file for complete search history.

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(57) **ABSTRACT**

A process for synthetically producing (S)-nicotine ([(S)-3-(1-methylpyrrolidin-2-yl) pyridine]) is provided.

14 Claims, No Drawings

Specification includes a Sequence Listing.

PROCESS OF MAKING (S)-NICOTINE

FIELD OF THE INVENTION

The present invention relates to a process for synthetically producing (S)-nicotine ([(S)-3-(1-methylpyrrolidin-2-yl) pyridine]).

BACKGROUND OF THE INVENTION

Nicotine (3-[1-methylpyrrolidin-2-yl]pyridine) is a natural product that may be obtained from the leaves of Nicotiana, i.e. the tobacco plant. There is considerable demand for nicotine products across the tobacco industry and also across the pharmaceutical field. For example, there remains a demand for traditional tobacco products e.g. traditional cigarettes, which is likely due to the addictive nature of nicotine. However, due to growing concern around the detrimental impact of traditional cigarette products on consumer health, there is an increasing demand for tobacco replacement products containing nicotine, such as electronic cigarette devices, patches, lozenges, nasal spray and chewing gum. Tobacco replacement products may be provided as a substitute for traditional tobacco products that would otherwise result in harmful carcinogenic effects; such as due to the presence of pyridine alkaloids, polycyclic aromatics, phenols and N-nitrosamines. Tobacco replacement products may be used specifically to treat nicotine dependence. Within the pharmaceutical field, there is also interest in the possible therapeutic applications of nicotine.

Challenges exist for obtaining nicotine with suitable levels of both enantiomeric purity and chemical purity. Nicotine is optically active, i.e. it may exist in one of two possible enantiomeric forms: (R)-nicotine or (S)-nicotine. Processes for obtaining racemic mixes of nicotine exist (e.g. WO2016065209). However, it is acknowledged that (S)-nicotine (i.e. [(S)-3-(1-methylpyrrolidin-2-yl) pyridine]) is significantly more active than (R)-nicotine. Therefore, the demand in the tobacco industry and in the pharmaceutical field is for nicotine with a high level of enantiomeric purity with respect to the (S) enantiomer. The pharmaceutical industry in particular imposes strict regulations on the required level of enantiomeric purity for new pharmaceutical products, and it is possible that the existing required level of enantiomeric purity for nicotine may increase. In addition to the demand for enantiomeric purity of nicotine, obtaining a high level of chemical purity is also of importance in both the pharmaceutical and tobacco industries—chemical purity referring to the amount of nicotine (i.e. both (R) and (S) enantiomeric forms) in comparison to non-nicotine impurities. The pharmaceutical industry already imposes very strict regulations on the required level of chemical purity of nicotine in comparison to non-nicotine impurities. In fact, the current U.S. Pharmacopeia reference standard for the chemical purity of nicotine is at least 99% with not more than 0.5% of any single impurity. A high chemical purity is also of significant importance to the tobacco industry, as the harmful carcinogenic effects mentioned above can be caused by impurities that are capable of exerting a carcinogenic effect.

(S)-Nicotine may be obtained by extraction from leaves of the tobacco plant. However, when nicotine is obtained this way, it typically has a chemical purity of less than 95% due of the presence of related alkaloid impurities. A typical composition of a nicotine sample obtained by extraction from tobacco leaves comprises 93% (S)-nicotine, 2.4% (S)-nornicotine, 3.9% (S)-anatabine and 0.5% (S)-anabasine

(E. Leete and M. Mueller, J. Am. Chem., Soc., 1982, 104, 6440-44). The alkaloid impurities are of a similar chemical structure to nicotine and consequently are difficult to remove. The actual composition of nicotine is also dependent on such factors as the geographic source and the season of harvest.

(S)-Nicotine may also be obtained by a synthetic process. There are various examples in the prior art for synthetically producing (S)-nicotine. For example, in the prior art are processes where a racemic (i.e. equal) mix of (R)-nicotine and (S)-nicotine is made, where this racemic mix is subsequently resolved to obtain the (S) enantiomer (U.S. Pat. No. 8,389,733, US 2014/0031554, and U.S. Pat. No. 8,378,111). There is also an example in the prior art of a synthetic process for producing (S)-nicotine using an enzyme as a biocatalyst (WO 2014/174505); the use of biocatalysts in enantiomerically selective processes in general are known outside of the nicotine field (L. S. Bleicher et al, *J. Org. Chem.,* 1998, 63, 1109-18, WO 2013/170050, WO2015/073555, P. N Scheller et al, *Chembiochem,* 2014, 15, 2201-4, Gand et al, *J Mol. Cat. B, Enzymatic,* 2014, 110, 126-32). Nevertheless, selectively synthesising (S)-nicotine in preference to the (R) enantiomer with high enantiomeric selectivity whilst also achieving high chemical purity remains a challenge.

SUMMARY OF THE INVENTION

In a first aspect, there is a process of making (S)-nicotine comprising the steps of:

(i) reducing myosmine with an enzyme with imine reductase activity to form (S)-nornicotine; and

(ii) methylating the (S)-nornicotine formed from step (i) to form (S)-nicotine.

It was surprisingly found that by way of steps (i) and (ii) of this process, where myosmine is used as the starting material, a very high enantiomeric and chemical purity was achieved for (S)-nicotine. This indicates that step (i) is a highly enantiomeric selective synthetic step with preference for the (S) isomer, and that step (ii) is such that this preference is retained in the final nicotine product, whilst also maintaining high chemical purity. This allows the production of (S)-nicotine without having to resort to resolution of a racemic mix. The high chemical purity is particularly advantageous; a reduced level of the undesirable impurities typically associated with nicotine results in a reduced risk of potential impurity-related negative effects. Furthermore, steps (i) and (ii) offer a convenient manufacturing process for making (S)-nicotine.

In a second aspect there is a process for producing a pharmaceutical composition, comprising forming (S)-nicotine using the process of the first aspect, and including the (S)-nicotine in the pharmaceutical composition together with one or more pharmaceutical excipients.

In a third aspect there is a process for producing a formulation for an electronic cigarette device, comprising forming (S)-nicotine using the process of the first aspect, and including the (S)-nicotine in a solvent with one or more additives.

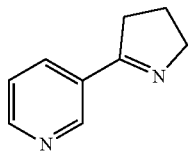
In a fourth aspect there is the use of myosmine and an enzyme with imine reductase activity in a process of forming (S)-nicotine.

In a fifth aspect there is a kit comprising myosmine and an enzyme with imine reductase activity, for use in the above process of forming (S)-nicotine.

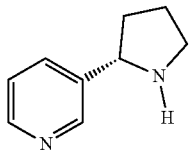
DESCRIPTION OF THE PREFERRED EMBODIMENTS

As the skilled person will appreciate, myosmine, (S)-nornicotine and (S)-nicotine, have the following structures:

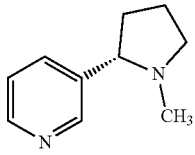
myosmine



(S)-nornicotine



(S)-nicotine



The skilled person will be familiar with appropriate reaction schemes to make myosmine.

As used herein, an "enzyme with imine reductase activity" refers to an enzyme capable of asymmetrically reducing an imine group, in particular a secondary imine group, to the corresponding amine group, in particular a secondary amine group. In particular, the enzyme with imine reductase activity used in the process disclosed herein is an enzyme capable of catalysing the conversion of myosmine to (S)-nornicotine. The skilled person is familiar with such enzymes. The enzyme may be added to the reaction mixture in a variety of forms, such as in the form of spray dried cells.

Preferably, the process uses an enzyme capable of converting myosmine to (S)-nornicotine such that the (S)-nornicotine is obtained with an enantiomeric excess of at least 90%, preferably at least 95%, more preferably at least 98%, most preferably at least 99%. Enantiomeric excess is measured in the manner given in the Examples. In the processes disclosed herein, this high enantiomeric excess is also achieved for the (S)-nicotine that is eventually achieved as the final product.

As the skilled person will appreciate, enzymes with imine reductase activity typically include NADH/NADPH dependent oxidoreductases, such as NADH/NADPH dependent dehydrogenases, and NADH/NADPH dependent imine reductases. NADH/NADPH dependent dehydrogenases include those referred to by enzyme classification number E.C.1.1.1, and include in particular 6-phosphogluconate dehydrogenases, referred to by enzyme classification number E.C.1.1.1.44. Imine reductases include those referred to with enzyme classification number E.C.1.5.1, in particular those referred to with enzyme classification number E.C 1.5.1.48.

Examples of different species of imine reductases include thiazolinyl imine reductase, dihydrofolate reductase, Δ^1—pyrroline-2-carboxylate reductase, Δ^1—piperideine-2-carboxylate reductase, sanguinarine reductase, and 1,2-dihydro reticuline reductase. Such enzymes can be isolated or derived from sources such as Streptomyces, Verrucosispora, Mesorhizobium, Yersinia, Pseudomonas, Candida albicans, Eschscholzia, and Papaver.

Examples of possible enzymes also include those disclosed in WO2013170050 (the contents of which are incorporated by reference).

The enzyme may be IRED_A, IRED_B, IRED_C, IRED_D, IRED_E, IRED_F, IRED_P, IRED_X, IRED_AB, IRED-20, or a homologue thereof. IRED_A, IRED_B, IRED_C, IRED_D, IRED_E, IRED_F, IRED_P, IRED_X, and IRED_AB are available from Enzymicals; IRED-20 is available from Almac Group. For example, in one embodiment, the enzyme is IRED_A, IRED_B, IRED_C, IRED_D, IRED_E, IRED-20, or a homologue thereof.

Disclosed herein, the enzyme may comprise an amino acid sequence according to any one of SEQ I.D. NO: 1, SEQ I.D. NO: 2, SEQ I.D. NO: 3, SEQ I.D. NO: 4, or a homologue thereof. In another embodiment, the enzyme comprises an amino acid sequence according to any one of SEQ I.D. NO: 1, SEQ I.D. NO: 2, SEQ I.D. NO: 3, or SEQ I.D. NO: 4.

As used herein, "a homologue thereof" means an enzyme comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of the enzymes disclosed herein. For example, "a homologue thereof" can mean an enzyme comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence according to any one of SEQ I.D. NO: 1, SEQ I.D. NO: 2, SEQ I.D. NO: 3, or SEQ I.D. NO: 4.

As used herein, the term "sequence identity" refers to a relationship between two or more amino acid sequences. When a position in one sequence is occupied by the same amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be "identical" at that position. The percentage "sequence identity" is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of "identical" positions. The number of "identical" positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of "sequence identity." Percentage of "sequence identity" is determined by comparing two optimally aligned sequences over a comparison window. In order to optimally align sequences for comparison, the portion of a polypeptide sequence in the comparison window may comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of "identical" positions between the reference and comparator sequences. Levels of sequence identity between coding sequences may be calculated using known methods.

The sequence identity can be calculated using publicly available computer-based methods for determining sequence identity including the BLASTP, BLASTN and FASTA (Atschul et al., J. Molec. Biol., 215: 403-410, (1990)), the BLASTX programme available from NCBI, and the Gap programme from Genetics Computer Group (Madison Wis.). Levels of sequence identity are obtained using the Gap programme, with a Gap penalty of 50 and a Gap length penalty of 3 for the amino acid sequence comparisons.

Generally, step (i) comprises reducing myosmine with the enzyme in the presence of a suitable cofactor, in particular NADH or NADPH. As the skilled person will appreciate, the enzyme and the cofactor may be introduced to the reaction

mixture as separate components, or they may be introduced to the reaction mixture as part of the same component for example in the form of whole microbial cells which contain both the enzyme and the appropriate cofactor. A suitable cofactor recycling system may be present to convert the cofactor from its oxidised form (NAD+ or NADP+) to its reduced form (NADH or NADPH). The skilled person will be familiar with appropriate cofactor recycling systems, such cofactor recycling systems including glucose(monohydrate)/glucose dehydrogenase, formate/formate dehydrogenase and isopropanol/alcohol dehydrogenase. When a cofactor recycling system is present, the cofactor may be added to the reaction mixture in its oxidised form i.e. as NAD+ or NADP+.

The cofactor itself may be present in the range of 0.02 parts to 10 parts by weight per 100 parts of myosmine. Preferably, the cofactor may be present in the range of 0.05 part to 5 parts by weight per 100 parts of myosmine. More preferably, the cofactor may be present in the range of 0.5 part to 2 parts by weight per 100 parts of myosmine.

The amount of enzyme present in step (i) can be present in an amount of 0.1 parts to 30 parts by weight per 100 parts of myosmine. Preferably, the amount of enzyme present in step (i) can be present in an amount of 0.5 parts to 10 parts by weight of myosmine. The skilled person will appreciate that the amount of enzyme present in step (i) can be tailored depending on the desired time period for the reaction of step (i), where more enzyme can be used for a shorter reaction time, and vice versa.

Step (i) may be carried out in the presence of an ion exchange resin, however preferably step (i) is carried out in absence of an ion exchange resin. The ion exchange resin, when present, is an Amberlite resin, an Amberlyst resin, an Amberjet resin, such as Amberlite IR-120, or a Dowex resin, where each of these ion exchange resins is available from Aldrich.

The possible pH for step (i) can be in the range of pH 5-9.

The (S)-nornicotine is converted to (S)-nicotine by a further step of: (ii) methylating the (S)-nornicotine formed from step (i) to form (S)-nicotine.

It was surprisingly found that following step (ii) the (S)-nicotine was achieved with particularly high chemical purity and particularly high enantiomeric excess.

The methylation step, i.e., step (ii), may be carried out by way of a muti-step process. For example, step (ii) may comprise forming a compound (e.g. N-formyl-(S)-nornicotine), and then subsequently reducing this compound to arrive at the methylated product i.e. (S)-nicotine. Preferably however, step (ii) is carried out by way of a single step process such as reductive methylation. As the skilled person will appreciate, the term "reductive methylation" refers to a process whereby a species is formed and reduced to arrive at the methylated product (i.e. (S)-nicotine) by way of a single step.

Preferably, the (S)-nornicotine is reductively methylated using formaldehyde or a formaldehyde-based compound. Step (ii) is particularly effective when using such reagents.

As used herein, a formaldehyde-based compound is used to refer to a compound that is capable of generating formaldehyde in-situ during a chemical reaction. The skilled person will appreciate that this means the formaldehyde-based compound is added to the reaction mixture, and then subsequently breaks down to release formaldehyde (and other related compounds) which may then react with the (S)-nornicotine to form (S)-nicotine. In the case of the addition of a formaldehyde-based compound, the skilled person will be familiar with how to tailor the appropriate amount of the formaldehyde-based compound added in order to achieve the release of a particular amount of formaldehyde in situ.

Formaldehyde itself has the formula HC(O)H and is generally introduced as a liquid or a gas. The formaldehyde may be introduced to the reaction mixture as part of an aqueous solution of formaldehyde (such aqueous solutions may be referred to as formalin).

The formaldehyde-based compound is generally introduced as a solid or a liquid. The formaldehyde-based compound may be a dimer of formaldehyde, a polymer of formaldehyde, or an acetal of formaldehyde. Preferably, the formaldehyde-based compound is a polymer of formaldehyde.

As the skilled person will appreciate, the term "polymer of formaldehyde" refers to a compound with three or more polymerised formaldehyde repeat units. Preferably, the polymer of formaldehyde is paraformaldehyde. As used herein, the term "paraformaldehyde" refers to polymer of formaldehyde with a degree of polymerization of 8-100 units.

When the (S)-nornicotine is reductively methylated using formaldehyde or a formaldehyde-based compound, the formaldehyde or formaldehyde-based compound may be added in an amount of 50 parts to 110 parts by weight, preferably 60 parts to 90 parts by weight, per 100 parts of (S)-nornicotine. Such amounts refer to the actual amounts of formaldehyde, formaldehyde-based compound and (S)-nornicotine present. Therefore, where for example the (S)-nornicotine is formed as part of a solution (e.g. an aqueous solution) and/or when the formaldehyde or formaldehyde-based compound is introduced to the reaction mixture as part of a solution (e.g. an aqueous solution) the parts by weight disclosed herein refer to the actual amounts of the formaldehyde, formaldehyde-based compound and (S)-nornicotine contained in the respective solutions.

Where the methylation step is a reductive methylation step, the reductant may be formic acid, sodium cyanoborohydride, or palladium/hydrogen, preferably formic acid. As the skilled person will appreciate, the appropriate amount of reductant will depend on the specific reductant used. For example, when the reductant is formic acid, the reductant may be present in an amount of 40-110 parts, preferably 40-100 parts, more preferably 50 parts to 70 parts by weight per 100 parts of (S)-nornicotine. Such amounts refer to the actual amounts of reductant and (S)-nornicotine present.

Preferably, steps (i) and (ii) may be carried out without isolating the (S)-nornicotine formed from step (i). This allows the formation of (S)-nicotine with both a high enantiomeric excess and a high chemical purity whilst using a particularly convenient synthetic route. Avoiding the need for isolation of the (S)-nornicotine from the reaction mixture formed from step (i) before converting this to (S)-nicotine has the benefit of offering a particularly convenient synthetic route, as isolation of the (S)-nornicotine can be process intensive as a result of costly plant time and energy (for example due to the need for large quantities of solvent for extraction and/or the boiling down of the solution). For example, in step (i) the (S)-nornicotine may be formed as part of an aqueous solution, where the aqueous solution containing the (S)-nornicotine is then carried through for direct use in step (ii). Consequently, the methylation step (step (ii)) is performed on the aqueous solution of (S)-nornicotine formed from step (i). When the process is carried out in this manner, it is preferable for the (S)-nornicotine to be reductively methylated either by using paraformaldehyde, or, by using formaldehyde that is intro-

duced to the reaction mixture as part of an aqueous solution. When the process is carried out in this manner, it is more preferable for the (S)-nornicotine to be reductively methylated by using formaldehyde that is introduced to the reaction mixture as part of an aqueous solution, as it has been found that this reduces undesirable frothing of the reaction mixture as the process proceeds.

The (S)-nicotine produced using the processes disclosed herein has an enantiomeric excess of at least 90%, preferably of at least 95%, more preferably of at least 98%, most preferably of at least 99%. The skilled person will be familiar with how to measure the enantiomeric excess. Enantiomeric excess may for instance be measured in the manner given in the Examples.

The (S)-nicotine produced using the processes disclosed herein has a chemical purity of at least 98%, preferably of at least 99%. The skilled person will be familiar with how to measure the chemical purity. Chemical purity may for instance be measured in the manner given in the Examples. The level of chemical purity achieved by the examples is particularly high.

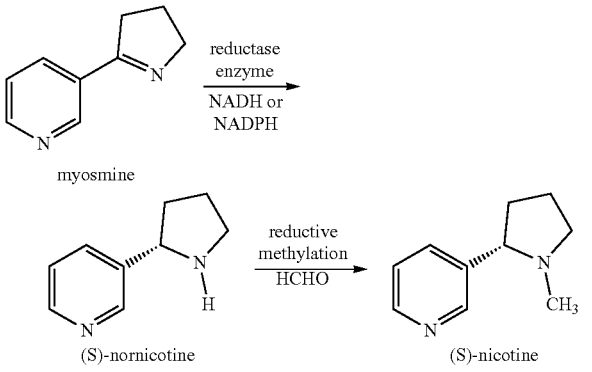
The (S)-nicotine produced using the method steps above may be included in a pharmaceutical composition together with one or more pharmaceutical excipients. Preferably, the pharmaceutical composition is a transdermal patch, a lozenge, or an inhalation formulation.

The (S)-nicotine produced using the method steps above may also be included in a formulation for inclusion in an electronic cigarette device. The formulation includes (S)-nicotine in a solvent with one or more additives. The solvent may comprise glycerol, propylene glycol, water, or mixtures thereof.

Preferably, the solvent comprises glycerol and propylene glycol, wherein the proportion of glycerol to propylene glycol is in the range of 80:20 to 20:80 by volume. The one or more additives may include one or more flavouring agents.

Also provided herein is a kit comprising myosmine and an enzyme with imine reductase activity for use a process of forming (S)-nicotine.

A particularly preferred reaction scheme is displayed below as scheme **1**:



The invention will be demonstrated with the following non-limiting examples.

EXAMPLES

The following examples demonstrate results associated with the process disclosed herein. Various reagents have been used to exemplify the process.

The enzymes used include the following:

IRED_A from Verrucosispora maris (strain AB-18-032, Uniprot:

F4F8G5_VERMA) with the amino acid sequence (a) or (b) given below—sequence (a) corresponds with SEQ I.D. NO: 1, and sequence (b) corresponds with SEQ I.D. NO: 2.

(a) As used with hexahistidine tag, total 302 amino acid residues:

MHHHHHHAADSRAPVTVIGLGAMGSALARAFLAAGHPTTVWNRSPDKA
DDLVGQGAVRAATVADAMSAGNLIVICVLDYRAMREIIDSTGHSPADR
VIVNLTSGTPGDARATAAWAQEQGMEYIDGAIMATPSMIGSEETLIFY
GGPQEVYDAHADTLRSIAGAGTYLGEEPGLPSLYDVALLGLMWTTWAG
FMHSAALLASEKVPAAAFLPYAQAWFEYVISPEVPNLATQVDTGAYPD
NDSTLGMQTVAIEHLVEASRTQGVDPTLPEFLHARAEQAIRRGHAGDG
FGAVFEVLRAPAAQ

(b) Original enzyme, total of 296 amino acid residues:

MAADSRAPVTVIGLGAMGSALARAFLAAGHPTTVWNRSPDKADDLVGQ
GAVRAATVADAMSAGNLIVICVLDYRAMREIIDSTGHSPADRVIVNLT
SGTPGDARATAAWAQEQGMEYIDGAIMATPSMIGSEETLIFYGGPQEV
YDAHADTLRSIAGAGTYLGEEPGLPSLYDVALLGLMWTTWAGFMHSAA
LLASEKVPAAAFLPYAQAWFEYVISPEVPNLATQVDTGAYPDNDSTLG
MQTVAIEHLVEASRTQGVDPTLPEFLHARAEQAIRRGHAGDGFGAVFE
VLRAPAAQ

IRED_B from Mesorhizobium sp. L48C026A00 aka a 6-phosphogluconate dehydrogenase, with the amino acid sequence (a) or (b) given below—sequence (a) corresponds with SEQ I.D. NO: 3, and sequence (b) corresponds with SEQ I.D. NO: 4.

(a) As used with hexahistidine tag, total 310 amino acid residues:

MHHHHHHASNVCVLGAGRMGSSIARTLLDRGYPTWVWNRTAAKCEPLA
ALGAKVASSVQEGIQAAEVVIINVLDYAASDALLKRDGIASALAGKAV
VQLTSGSPRLAREEARWVEAHGAGYLDGAIMATPDFIGKPETAMLYSG
SRDVYEKHKPLLFALGGGTNYVGELPGQASALDTALLTQMWGGLFGAL
QGMAVAEAEGLDLETFRNHLSAFKPVVDASLFDLVDRTNARRFAGDDA
TLASLGAHYSAFQHLLEACEERGLDAAMPRAMDMIFRQALSLGSMEDD
LASLALLFRNGSPRQSREPANA

(b) Original enzyme, total of 304 amino acid residues

MASNVCVLGAGRMGSSIARTLLDRGYPTWVWNRTAAKCEPLAALGAKV
ASSVQEGIQAAEVVIINVLDYAASDALLKRDGIASALAGKAVVQLTSG
SPRLAREEARWVEAHGAGYLDGAIMATPDFIGKPETAMLYSGSRDVYE

-continued

KHKPLLFALGGGTNYVGELPGQASALDTALLTQMWGGLFGALQGMAVA

EAEGLDLETFRNHLSAFKPVVDASLFDLVDRTNARRFAGDDATLASLG

AHYSAFQHLLEACEERGLDAAMPRAMDMIFRQALSLGSMEDDLASLAL

LFRNGSPRQSREPANA

Example 1

Biotransformations were undertaken at 0.5 mL scale with a solution of 10 mM myosmine and NADP+(0.5 mM), glucose (25 mM), glucose dehydrogenase (10 U/ml), and the enzyme with imine reductase activity. The enzymes used are detailed in table 1, available from Enzymicals. For each enzyme, the amount of enzyme was 9 mg/ml of cell free extract (estimated approx. 0.9 mg/ml contained enzyme). For IRED_B and IRED_C specifically, additional tests were run which used 0.9 mg/ml cell free extract.

The enantiomeric excess of the (S) nornicotine obtained from the biotransformation was determined using a Chiralpak AD-H column (250×4.6 mm id) eluting with a mixture of hexane:ethanol:diethylamine 74.9:25.0:0.1 (v/v/v) at 1 ml/min over 18 min at 30° C. This method was also used to measure the conversion of myosmine into nornicotine, a relative response factor of 2.18:1 having been determined for uv absorption detection at 254 nm.

The results are displayed in table 1 below.

TABLE 1

	Enzyme	Amount	Conversion [%]	Enantiomeric Excess [% S]
i	IRED_A	9 mg/ml	99.3	99.8
ii	IRED_B	9 mg/ml	99.9	98.4
iii	IRED_B	0.9 mg/ml	99.3	98.4
iv	IRED_C	9 mg/ml	99.3	92.9
v	IRED_C	0.9 mg/ml	100.0	99.1
vi	IRED_D	9 mg/ml	99.6	99.8
vii	IRED_E	9 mg/ml	99.4	99.8
viii	IRED_F	9 mg/ml	99.1	86.5
ix	IRED_P	9 mg/ml	97.7	86.6
x	IRED_X	9 mg/ml	99.8	95.7
xi	IRED_AB	9 mg/ml	99.6	96.8

The % enantiomeric excess for (S)-nornicotine was identified according to the equation [(S)−(R)]/((S)+(R)]×100 where (S) and (R) are the amounts of (S) and (R) enantiomers present respectively. The % conversion was identified according to the amount of myosmine consumed i.e. according to the equation 100−(final amount of myosmine)/(starting amount of myosmine)×100.

Example 2

Reactions were carried out in a similar manner to that of example 1, except that 1.5 equivs glucose and 1 mol % NADP+ were used relative to the myosmine substrate, and a 24 hr reaction time was employed. The enzymes used are detailed in each of tables 2, 3 and 4 (available from Enzymicals).

At 100 mM myosmine concentration, using 0.9 mg/mL enzyme cell free extract, the results were as displayed in the table below:

TABLE 2

	Enzyme	Conversion [A]	Enantiomeric Excess [% S]
i	IRED_A	63.6	99.8
ii	IRED_B	99.9	98.7
iii	IRED_C	99.9	99.8
iv	IRED_D	99.0	99.9
v	IRED_E	99.9	99.9

At 100 mM myosmine concentration, using 9 mg/mL enzyme cell free extract, the results were as displayed in the table below:

TABLE 3

	Enzyme	Conversion [A]	Enantiomeric Excess [% S]
i	IRED_A	99.9	99.8
ii	IRED_B	99.8	98.8
iii	IRED_C	99.8	99.9
iv	IRED_D	99.9	100.0
v	IRED_E	99.9	99.9

At 250 mM myosmine concentration, using 9 mg/mL enzyme cell free extract, the results were as displayed in the table below:

TABLE 4

	Enzyme	Conversion [A]	Enantiomeric Excess [% S]
i	IRED_A	100.0	99.7
ii	IRED_B	99.9	98.6
iii	IRED_C	100.0	99.9
iv	IRED_D	100.0	99.9
v	IRED_E	99.9	99.9

Example 3

A solution of myosmine (20 mmol, 2.924 g), D-Glucose (30 mmol, 5.405 g) nicotinamide adenine dinucleotide phosphate sodium salt (0.2 mmol, 157 mg), enzyme IRED_A (available from Enzymicals) cell free extract lyophilizate (1.0 g), glucose dehydrogenase (2000 U, 40 mg) in pH7.5 100 mM sodium phosphate buffer (200 mL) was mixed by an overhead stirrer at 200 rpm at 30° C. for 24 hours. The solution was analysed for nornicotine during the course of the reaction with HPLC showing 77% conversion after 8 hours, and over 99% conversion after 24 h with 98.7% e.e. (S)-Nornicotine. This solution was then treated with 37% formaldehyde solution (8.1 g) and formic acid (2.8 g) at 80° C. for 4 h, with the reaction being complete after 2 h. After cooling, 6 g solid sodium hydroxide was added (pH 12.7) and the mixture extracted with 2×75 ml MTBE. After drying over sodium sulphate, the solvent was removed to afford 2.25 g crude (S)-nicotine which was >99% pure by HPLC (area % at 260 nm) and had 98.7% enantiomeric excess.

Example 4

A solution of myosmine (20 mmol, 2.924 g), D-Glucose (30 mmol, 5.405 g) nicotinamide adenine dinucleotide phosphate sodium salt (0.2 mmol, 157 mg), enzyme IRED_B (available from Enzymicals) cell free extract lyophilizate (0.5 g), glucose dehydrogenase (2000 U, 40 mg) in pH7.5 100 mM sodium phosphate buffer (200 mL) was mixed by

overhead stirrer at 200 rpm at 30° C. for 24 hours. The solution was analysed for nornicotine during the course of the reaction with HPLC showing 91% conversion after 4 hours, and over 99% conversion after 6 hours. After 24 hours the (S)-Nornicotine was 98.2% e.e. This solution was then treated with paraformaldehyde (3 g) and formic acid (2.8 g) at 80° C. for 6 h, with the reaction being complete after 4 h. After cooling, 6 g solid sodium hydroxide was added (pH 12.7) and the mixture extracted with 2×75 ml MTBE. After drying over sodium sulphate, the solvent was removed to afford 2.31 g crude (S)-nicotine which was >99% pure by HPLC (area % at 260 nm) and had 98.3% enantiomeric excess.

Example 5

This example demonstrates the enantioselectivity and conversion rate at high substrate concentrations. This example was carried out in a similar manner to example 1 except that all reactions used glucose 1.5 equivs, NADP (1% relative to the myosmine), imine reductase, specifically IRED_C available from Enzymicals (4.5 mg/ml cell free extract, GDH (10 U/ml per 250 mM of myosmine concentration), sodium phosphate buffer pH7.5 100 mM over a 24 hour time period. The results are shown below.

TABLE 5

	Concentration of myosrnine starting material	Conversion [%]	Enantiomeric Excess [% S]
i	250 mM	99.9	99.7
ii	400 mM	99.6	99.8
iii	600 mM	68.8	99.8
iv	800 mM	56.5	99.7
v	1000 mM	52.4	99.6

Example 6

This example demonstrates the enantioselectivity and conversion rate on a larger scale.

A solution of myosmine (400 mmol, 58.5 g), D-Glucose (600 mmol, 118.9 g) nicotinamide adenine dinucleotide phosphate sodium salt (4 mmol, 3.15 g), enzyme IRED_C (available from enzymicals) cell free extract lyophilisate (10.0 g), glucose dehydrogenase CFE (0.32 g) in pH7.5 100 mM sodium phosphate buffer (1000 mL) was mixed with an overhead stirrer at 200 rpm at 30° C. for 24 hours. The solution was analysed for nornicotine after 24 hours with HPLC and showed over 98% conversion.

Details of the workup are as follows: the biocatalytic reaction mixture was acidified with concentrated sulphuric acid to pH 1-2, then heated to 90° C. for 20 minutes to precipitate all the proteins. Proteins were filtered out of the mixture over Celite. The resulting clear solution was basified with 40% NaOH solution to pH>11 and extracted four times with 500 mL methyl tert-butyl ether (MTBE). The combined MTBE phases were dried over anhydrous magnesium sulfate and the solvent evaporated. The isolated yield of nornicotine was 41.1 g (70%) as a brown-yellow liquid.

A separate sample of the nornicotine reaction mixture prior to work up and isolation was taken through to the methylation step. Specifically, without isolation of the nornicotine, to the biocatalytic reaction mixture, paraformaldehyde (60 g) and formic acid (49.2 g) were added. The reaction was heated to 85° C. and stirred vigorously to form (S)-nicotine.

Example 7

The general experimental method to form (S)-nicotine was as follows. Biocatalysis of myosmine into (S)-nornicotine using IRED_C (available from Enzymicals) was conducted at a concentration of 400 mM myosmine. Either the (S)-nornicotine was isolated by way of extraction with methyl-tert butyl ether and removal of the solvent, or the aqueous solution from the biocatalysis was heated at 90° C. for 15 min to precipitate proteins, then after cooling the mixture was acidified to pH 1-2 with sulfuric acid, the precipitated protein removed by filtration through Celite, and the solution then neutralised with aqueous sodium hydroxide to about pH7.

Example 7a

Crude isolated nornicotine from the enzyme reduction of myosmine (92 g) was added to 800 ml water. Paraformaldehyde (74 g, 4 eq) and formic acid (58 g, 2 eq) were added. The mixture was gradually warmed to 80-85 degrees C. HPLC analysis after 2 h indicated completion of the reaction. The mixture was kept at the same temperature for a further 2 h and then cooled to room temperature. 50% Sodium hydroxide solution was added to obtain a pH of approximately 13. The mixture was extracted with 2×500 ml MTBE and dried over sodium sulphate. The solvent was removed and the crude (S)-nicotine distilled under vacuum. After a forerun of about 4 g, 87 g of purified nicotine was obtained (>99% by HPLC and 99.6% ee by chiral HPLC).

Example 7b

To 2.5 litres of aqueous nor-nicotine solution from the same biocatalysis as used in Example 1 (5.63 g/100 ml) was added paraformaldehyde (112.5 g, 4 eq) and formic acid (88 g, 2 eq). The mixture was gradually heated to 80-85 degrees C., with reaction beginning at about 70 degrees C. with some foaming due to gas evolution. After 1 h at 80-85 degrees C., HPLC indicated the reaction to be complete. The reaction was heated for 4 h in total and then cooled. The mixture was basified with 50% sodium hydroxide solution and extracted with MTBE (800 ml then 500 ml). After drying, the crude mixture was distilled to give 118.7 g (S)-nicotine (>99% by HPLC and 99.5% ee by HPLC).

Example 7c

To 2.5 litres of aqueous nor-nicotine solution from the same biocatalysis as used in Example 1 (5.63 g/100 ml) was added 37% formaldehyde solution (290 ml, ~4 eq) and formic acid (88 g, 2 eq). The mixture was gradually heated to 80-85 degrees C., with reaction beginning at about 60 degrees C. with some foaming due to gas evolution. After 1 h at 80-85 degrees C., HPLC indicated the reaction to be complete. The reaction was heated for 4 h in total and then cooled. The mixture was basified with 50% sodium hydroxide solution and extracted with MTBE (800 ml then 500 ml). After drying, the crude mixture was distilled to give 119.1 g (S)-nicotine (>99% by HPLC and 99.5% ee by HPLC).

Example 8

A solution of myosmine (298 g) and glucose monohydrate (505 g) was made in 0.1M dipotassium hydrogen phosphate buffer (6 L). Amberlite IR-120 resin (2 kg, wet) was added as the ion exchange resin and the solution adjusted to pH7

with 12M sodium hydroxide (about 0.3 L), then stirred overnight at 25° C. to ensure a stable pH. Glucose dehydrogenase GDH-102 (6 g), beta-NADP+(6 g), and enzyme IRED-20 available from the Almac Group (30 g) were added, then the mixture stirred at 150 rpm while held at 25° C. with the pH maintained in the range 6.8-7.0 through additions of 4M postassium hydroxide. After 72 h the solution was decanted and the Amberlite resin was washed with deionized water (3×3 L). Then Amberlite resin was transferred to a column and further washed with deionized water (4 L), then shaken for 3 hours with 2M ammonia solution (4 L) and further washed with 2M ammonia (10 L). The combined solutions were concentrated under reduced pressure to dryness to give (S)-Nornicotine (131.2 g) as a yellow liquid. In order to recover further nornicotine out of the reaction mixture, reactivated Amberlite resin (2 kg) was added to it and the mixture stirred overnight at room temperature. The same treatment was repeated as above to recover further (S)-Nornicotine (59.8 g) bringing the total yield to 191.0 g. The above two batches were converted into (S)-nicotine separately. For the larger batch the (S)-nornicotine (126.2 g of) was combined with paraformaldehyde (154.5 g) and formic acid (118 g) in water (1 L) and the resulting stirred mixture heated to 85° C. overnight. The mixture was then cooled to 0° C., and adjusted to pH 14 with 12M sodium hydroxide. The mixture was extracted with methyl tert-butyl ether (3×8 vols). The organic phase was dried with anhydrous magnesium sulfate and concentrated to dryness to give crude (S)-nicotine as a yellow liquid (131.2 g). The second batch of (S)-nornicotine (59.8 g) had likewise been transformed into crude (S)-nicotine (60.7 g) in the same manner making a total yield of crude nicotine of 191.9 g. These were combined and distilled under reduced pressure (b.p. 70-77° C. at 0.53-0.67 mbar) to provide (S)-nicotine (174.5 g) as a colourless liquid with an enantiomeric excess of 99.38% as determined by HPLC, and a chemical purity of 99.96%, as determined by HPLC. Further of the process used to measure enantiomeric excess and chemical purity are given below.

Enantiomeric purity by HPLC: using a Chiracel OD-H column eluting with n-hexane and 1-butanol in a ratio of 95:5 and containing 0.1% diethylamine. The (R)-enantiomer eluted at 6.1 min and the (S)-enantiomer at 5.6 min. The enantiomeric excess is determined from the area of the peaks identified according to the equation [(S)−(R)]/((S)+(R)]. The enantiomeric excess was thus determined as 99.38%.

Chemical purity by HPLC: Using an X-Bridge C18 column with an eluant comprising a mixture of (i) 20 mM ammonium bicarbonate in water (pH=8.7) and (ii) acetonitrile in a gradient programme of 0-10 mins at 95:5, 10-13 mins at 70:30; 13-16 mins at 10:90; and subsequently 95:5. Temperature was 35 degrees C. The conditions of the detector were of UV absorption at a wavelength of 260 nm. A single impurity at 12.132 minutes at 0.04% area was found versus nicotine at 9.925 mins. With a single impurity at 0.04% area the purity was deemed as 99.96%. In comparison, prior to distillation the weighted average of the two batches used was 99.70%.

SEQUENCE LISTING

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<213> ORGANISM: Verrucosispora maris

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            20                  25                  30

Ala Ala Gly His Pro Thr Thr Val Trp Asn Arg Ser Pro Asp Lys Ala
        35                  40                  45

Asp Asp Leu Val Gly Gln Gly Ala Val Arg Ala Ala Thr Val Ala Asp
    50                  55                  60

Ala Met Ser Ala Gly Asn Leu Ile Val Ile Cys Val Leu Asp Tyr Arg
65                  70                  75                  80

Ala Met Arg Glu Ile Ile Asp Ser Thr Gly His Ser Pro Ala Asp Arg
                85                  90                  95

Val Ile Val Asn Leu Thr Ser Gly Thr Pro Gly Asp Ala Arg Ala Thr
            100                 105                 110

Ala Ala Trp Ala Gln Glu Gln Gly Met Glu Tyr Ile Asp Gly Ala Ile
        115                 120                 125

Met Ala Thr Pro Ser Met Ile Gly Ser Glu Glu Thr Leu Ile Phe Tyr
    130                 135                 140

Gly Gly Pro Gln Glu Val Tyr Asp Ala His Ala Asp Thr Leu Arg Ser
145                 150                 155                 160

Ile Ala Gly Ala Gly Thr Tyr Leu Gly Glu Glu Pro Gly Leu Pro Ser
                165                 170                 175
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Leu Tyr Asp Val Ala Leu Leu Gly Leu Met Trp Thr Thr Trp Ala Gly
            180                 185                 190

Phe Met His Ser Ala Ala Leu Leu Ala Ser Glu Lys Val Pro Ala Ala
        195                 200                 205

Ala Phe Leu Pro Tyr Ala Gln Ala Trp Phe Glu Tyr Val Ile Ser Pro
    210                 215                 220

Glu Val Pro Asn Leu Ala Thr Gln Val Asp Thr Gly Ala Tyr Pro Asp
225                 230                 235                 240

Asn Asp Ser Thr Leu Gly Met Gln Thr Val Ala Ile Glu His Leu Val
                245                 250                 255

Glu Ala Ser Arg Thr Gln Gly Val Asp Pro Thr Leu Pro Glu Phe Leu
            260                 265                 270

His Ala Arg Ala Glu Gln Ala Ile Arg Arg Gly His Ala Gly Asp Gly
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Met Gly Ser Ala Leu Ala Arg Ala Phe Leu Ala Ala Gly His Pro Thr
            20                  25                  30

Thr Val Trp Asn Arg Ser Pro Asp Lys Ala Asp Asp Leu Val Gly Gln
        35                  40                  45

Gly Ala Val Arg Ala Ala Thr Val Ala Asp Ala Met Ser Ala Gly Asn
    50                  55                  60

Leu Ile Val Ile Cys Val Leu Asp Tyr Arg Ala Met Arg Glu Ile Ile
65                  70                  75                  80

Asp Ser Thr Gly His Ser Pro Ala Asp Arg Val Ile Val Asn Leu Thr
                85                  90                  95

Ser Gly Thr Pro Gly Asp Ala Arg Ala Thr Ala Ala Trp Ala Gln Glu
            100                 105                 110

Gln Gly Met Glu Tyr Ile Asp Gly Ala Ile Met Ala Thr Pro Ser Met
        115                 120                 125

Ile Gly Ser Glu Glu Thr Leu Ile Phe Tyr Gly Gly Pro Gln Glu Val
    130                 135                 140

Tyr Asp Ala His Ala Asp Thr Leu Arg Ser Ile Ala Gly Ala Gly Thr
145                 150                 155                 160

Tyr Leu Gly Glu Glu Pro Gly Leu Pro Ser Leu Tyr Asp Val Ala Leu
                165                 170                 175

Leu Gly Leu Met Trp Thr Thr Trp Ala Gly Phe Met His Ser Ala Ala
            180                 185                 190

Leu Leu Ala Ser Glu Lys Val Pro Ala Ala Ala Phe Leu Pro Tyr Ala
        195                 200                 205

Gln Ala Trp Phe Glu Tyr Val Ile Ser Pro Glu Val Pro Asn Leu Ala
    210                 215                 220

Thr Gln Val Asp Thr Gly Ala Tyr Pro Asp Asn Asp Ser Thr Leu Gly
225                 230                 235                 240

Met Gln Thr Val Ala Ile Glu His Leu Val Glu Ala Ser Arg Thr Gln
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               245                 250                 255

Gly Val Asp Pro Thr Leu Pro Glu Phe Leu His Ala Arg Ala Glu Gln
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Val Leu Arg Ala Pro Ala Ala Gln
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            20                  25                  30

Pro Thr Trp Val Trp Asn Arg Thr Ala Ala Lys Cys Glu Pro Leu Ala
        35                  40                  45

Ala Leu Gly Ala Lys Val Ala Ser Ser Val Gln Glu Gly Ile Gln Ala
    50                  55                  60

Ala Glu Val Val Ile Ile Asn Val Leu Asp Tyr Ala Ala Ser Asp Ala
65                  70                  75                  80

Leu Leu Lys Arg Asp Gly Ile Ala Ser Ala Leu Ala Gly Lys Ala Val
                85                  90                  95

Val Gln Leu Thr Ser Gly Ser Pro Arg Leu Ala Arg Glu Glu Ala Arg
            100                 105                 110

Trp Val Glu Ala His Gly Ala Gly Tyr Leu Asp Gly Ala Ile Met Ala
        115                 120                 125

Thr Pro Asp Phe Ile Gly Lys Pro Glu Thr Ala Met Leu Tyr Ser Gly
    130                 135                 140

Ser Arg Asp Val Tyr Glu Lys His Lys Pro Leu Leu Phe Ala Leu Gly
145                 150                 155                 160

Gly Gly Thr Asn Tyr Val Gly Glu Leu Pro Gly Gln Ala Ser Ala Leu
                165                 170                 175

Asp Thr Ala Leu Leu Thr Gln Met Trp Gly Gly Leu Phe Gly Ala Leu
            180                 185                 190

Gln Gly Met Ala Val Ala Glu Ala Glu Gly Leu Asp Leu Glu Thr Phe
        195                 200                 205

Arg Asn His Leu Ser Ala Phe Lys Pro Val Val Asp Ala Ser Leu Phe
    210                 215                 220

Asp Leu Val Asp Arg Thr Asn Ala Arg Arg Phe Ala Gly Asp Asp Ala
225                 230                 235                 240

Thr Leu Ala Ser Leu Gly Ala His Tyr Ser Ala Phe Gln His Leu Leu
                245                 250                 255

Glu Ala Cys Glu Glu Arg Gly Leu Asp Ala Ala Met Pro Arg Ala Met
            260                 265                 270

Asp Met Ile Phe Arg Gln Ala Leu Ser Leu Gly Ser Met Glu Asp Asp
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35 40 45

Ala Ser Ser Val Gln Glu Gly Ile Gln Ala Ala Glu Val Val Ile Ile
50 55 60

Asn Val Leu Asp Tyr Ala Ala Ser Asp Ala Leu Leu Lys Arg Asp Gly
65 70 75 80

Ile Ala Ser Ala Leu Ala Gly Lys Ala Val Val Gln Leu Thr Ser Gly
85 90 95

Ser Pro Arg Leu Ala Arg Glu Glu Ala Arg Trp Val Glu Ala His Gly
100 105 110

Ala Gly Tyr Leu Asp Gly Ala Ile Met Ala Thr Pro Asp Phe Ile Gly
115 120 125

Lys Pro Glu Thr Ala Met Leu Tyr Ser Gly Ser Arg Asp Val Tyr Glu
130 135 140

Lys His Lys Pro Leu Leu Phe Ala Leu Gly Gly Gly Thr Asn Tyr Val
145 150 155 160

Gly Glu Leu Pro Gly Gln Ala Ser Ala Leu Asp Thr Ala Leu Leu Thr
165 170 175

Gln Met Trp Gly Gly Leu Phe Gly Ala Leu Gln Gly Met Ala Val Ala
180 185 190

Glu Ala Glu Gly Leu Asp Leu Glu Thr Phe Arg Asn His Leu Ser Ala
195 200 205

Phe Lys Pro Val Val Asp Ala Ser Leu Phe Asp Leu Val Asp Arg Thr
210 215 220

Asn Ala Arg Arg Phe Ala Gly Asp Asp Ala Thr Leu Ala Ser Leu Gly
225 230 235 240

Ala His Tyr Ser Ala Phe Gln His Leu Leu Glu Ala Cys Glu Glu Arg
245 250 255

Gly Leu Asp Ala Ala Met Pro Arg Ala Met Asp Met Ile Phe Arg Gln
260 265 270

Ala Leu Ser Leu Gly Ser Met Glu Asp Asp Leu Ala Ser Leu Ala Leu
275 280 285

Leu Phe Arg Asn Gly Ser Pro Arg Gln Ser Arg Glu Pro Ala Asn Ala
290 295 300

The invention claimed is:

1. A process of making (S)-nicotine comprising the steps of:

(i) reducing myosmine with an enzyme with imine reductase activity to form (S)-nornicotine; and

(ii) methylating the (S)-nornicotine formed from step (i) to form (S)-nicotine;

wherein step (ii) is carried out by way of reductive methylation; and

wherein in step (ii) the (S)-nornicotine is reductively methylated, using formaldehyde or a formaldehyde-based compound in the presence of a reductant.

2. The process of claim **1** wherein the formaldehyde is introduced as part of an aqueous solution.

3. The process of claim **1** wherein the formaldehyde-based compound is a dimer of formaldehyde, a polymer of formaldehyde, or an acetal of formaldehyde.

4. The process of claim **1**, wherein the reductant is formic acid, sodium cyanoborohydride, or palladium/hydrogen.

5. The process of claim 1, wherein the reductant is formic acid.

6. The process of claim 1, wherein the process is carried out without isolation of the (S)-nornicotine formed from step (i).

7. The process of claim 1, wherein in step (i) the (S)-nornicotine is formed as part of an aqueous solution, and wherein step (ii) comprises methylating the (S)-nornicotine contained within the aqueous solution.

8. The process according to claim 7, wherein in step (ii) the (S)-nornicotine is reductively methylated using formaldehyde introduced as part of an aqueous solution.

9. The process of claim 1, wherein the (S)-nicotine is obtained with an enantiomeric excess of at least 90%, preferably at least 95%, more preferably at least 98%, most preferably at least 99%.

10. A process for producing a pharmaceutical composition, comprising forming (S)-nicotine according to the process of claim 1, and including the (S)-nicotine in the pharmaceutical composition together with one or more pharmaceutical excipients.

11. The process of claim 10, wherein the pharmaceutical composition is a transdermal patch, a lozenge, or an inhalation formulation.

12. A process for producing a formulation for an electronic cigarette device, comprising forming (S)-nicotine according to the process of claim 1, and including the (S)-nicotine in a solvent with one or more additives.

13. The process of claim 2, wherein the reductant is formic acid, sodium cyanoborohydride, or palladium/hydrogen.

14. The process of claim 2, wherein the reductant is formic acid.

* * * * *

EXHIBIT 3

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20 December 2021

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By email to: cathy@hkhangsen.com; service@hangsen.com; service@hangsentech.com; yyzhang@cnhanxing.com

Attention: Directors and Legal Department

Dear Hangsen

Infringement of Zanoprima patents (EU patent number EP3653617 (UK designation) and US patent number 10913962) by Hangsen International Group. Notice to cease importing, selling, and offering Synthetic S-Nicotine products

We act for Zanoprima Lifesciences Limited (**Zanoprima**). Zanoprima is a global research-driven life sciences company based in the UK. It is a leader in the movement towards a tobacco-free world, and through its investment and innovation in respect of synthetic (S)-Nicotine, it has created an enzyme-based process for preparing (S)-Nicotine from myosmine that results in a totally synthetic, high purity product. This process is a patented process.

For the reasons set out in this letter, Zanoprima believes that Hangsen International Group Ltd, either in its own right or through its subsidiaries and associated group entities (**Hangsen**) is knowingly infringing Zanoprima's patents, not least through making available Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use Hangsen MOTiVO™ Synthetic S-Nicotine in the UK and US markets. The importation and disposal of these products in these jurisdictions cannot continue, and should Hangsen fail to comply with the requests set out in this letter, Zanoprima reserves its right to commence proceedings without further notice.

1. Background

1.1 We understand that "MOTIVO" is a trade mark registered with the United States Patent and Trademark Office (**USPTO**) to Jasper Technology, LLC (**Jasper Technology**). Amongst other things, the trade mark is registered in respect of a class of trade marks that covers electronic cigarettes and their parts. News reports suggest that since registering the "MOTIVO" trade mark, Jasper Technology has merged into Hangsen, and is now known as the Hangsen USA branch.

1.2 We also understand that Vision East Technology (Shenzhen) Co., Limited (**Vision East**), a Chinese company headquartered in Shenzhen, applied for registration of the figurative mark MOTiVO in class 34 with the European Union Intellectual Property Office (**EUIPO**) on or around 30 September 2020. Unsurprisingly, class 34 relates to e-cigarettes and their parts, and includes things such as flavourings and liquid nicotine solutions. Zanoprima is aware of the close relationship that Hangsen has with Vision East, and knows that Hangsen has collaborated with Vision East in respect of a number of patent filings over the years.

1.3 The MOTiVO™ brand is something that is clearly associated with Hangsen, and the "MOTIVO" trade mark has been used in the industry to describe a "patented synthetic nicotine". To this end, we note that in response to the question "[w]hat is MOTiVO™?", Hangsen's own website (available at https://www.hangsentech.com/) (the **Website**) provides:

> *Hangsen MOTiVO™ Synthetic S-Nicotine uses innovative enzymatic catalysis technology. Purity up to 99% or more, providing consumers incomparable satisfaction that racemic nicotine can't offer, also significantly reducing harmful impurities for vapers.*

The Website then goes on to provide a handful of reasons as to why consumers should choose "MOTiVO™", and provides a click through link where consumers with access to the Website can "order now".

1.4 Notwithstanding Hangsen's association with the MOTiVO™ branding, Hangsen MOTiVO™ Synthetic S-Nicotine products are not the only products sold that use Hangsen Synthetic S-Nicotine. Genmist by Nioo Labs is marketing Genmist branded nicotine pouches and heatsticks that are stated to contain the "patented synthetic nicotine Motivo" (the **Genmist Products**), and Zanoprima is aware that Hangsen is white labelling Hangsen MOTivO™ Synthetic S-Nicotine and allowing it to be re-branded by importers in the US.

2. Zanoprima's patents

2.1 Zanoprima is the registered proprietor of patents regarding the process of preparing (S)-Nicotine from myosmine. Zanoprima refers to the (S)-Nicotine derived from its process as "SyNic". SyNic was the world's first totally synthetic (S)-Nicotine not obtained from any part of tobacco or derived from a synthetic racemic mixture.

2.2 Zanoprima's patented process is based on a biochemical transformation. Using the steps of a reduction of myosmine to (S)-nornicotine catalysed by an enzyme called an imine reductase, followed by a methylation of the (S)-nornicotine, SyNic is produced directly in the (S)-isomer form. Zanoprima's patent in respect of this enzyme-based process has been granted in a number of jurisdictions, including the UK (European patent number 3653617) and US (US patent number 10913962) (the **Zanoprima Patents**), and is pending in many others, including China (Chinese patent number 113272289). We hereby put you

on notice that, on grant of its Chinese patent, Zanoprima would be entitled to take action against Hangsen in China in respect of any infringements of its patent rights in that jurisdiction also.

2.3 Details of the Zanoprima Patents are enclosed.

3. The infringing conduct

3.1 In addition to publically backing Nioo Labs, and it being clear to Zanoprima that Hangsen is the company effectively behind and in control of Genmist Products, Zanoprima is aware that Hangsen has partnerships and associations with the Jincheng group of companies (including Shandong Jincheng Medicine Chemical Co Ltd) (the **Jincheng Group**) and that:

3.1.1 a patent with the application number CN112409327A was filed by a Shandong Jincheng Medicine Chemical Co Ltd (**Jincheng**) and Hangsen on 18 November 2020. This application related to an enzyme-based process to (S)-Nicotine that was identical to the process outlined in the Zanoprima Patents. Unsurprisingly, the application was rejected by the Chinese Examiner because neither the independent or dependent claims made as part of the patent application were innovative; and

3.1.2 a patent with the application number CN112795603A was then filed by Jincheng and Hangsen on 14 December 2020. This application relates to a process to produce (S)-nornicotine and the second claim specifies *Myxococcus fulvus* as the source of imine reductase. In this regard, Zanoprima notes:

3.1.2.1 While the Zanoprima Patents relate to a process to produce (S)-Nicotine, they cite in their examples the imine reductase mediated reduction of myosmine to (S)-nornicotine; and

3.1.2.2 The choice of imine reductase from *Myxococcus fulvus* lacks any inventive step.

3.2 Irrespective of Zanoprima's position on the validity of CN112795603A, there is no significant market for (S)-nornicotine. The only practical use of (S)-nornicotine would be as an intermediate to make (S)-Nicotine, and if the process outlined in CN112795603A was to be taken through to the production of (S)-Nicotine, this would be an unauthorised use of Zanoprima's patented process.

3.3 Coupling the above with the fact that the Jincheng Group has issued a number of press releases about it having obtained approval for manufacturing nicotine using a biotransformation followed by a chemical step, it is obvious to Zanoprima that Hangsen MOTiVO™ Synthetic S-Nicotine, and any products that use Hangsen MOTiVO™ Synthetic S-Nicotine (including the Genmist Products and any white labelled products being sold under a different company's brand), are (a) products that have been developed in association with the Jincheng Group, and (b) are products that have either been developed in accordance with the process outlined in CN112409327A, or developed in accordance with the process outlined in CN112795603A, albeit taken through to (S)-Nicotine. In this regard, Zanoprima notes:

3.3.1 On the Website, Hangsen claims that "*Hangsen MOTiVO™ Synthetic S-Nicotine uses innovative enzymatic catalysis technology*" and has a "*[p]urity up to 99% or more*". CN112409327A is the only patent application associated with Hangsen that uses an enzymatic process. CN112409327A was rejected as it was identical to the Zanoprima Patents.

3.3.2 If the process outlined in CN112409327A is not being followed, then the process outlined in CN112795603A taken through to (S)-Nicotine is. This inference can be drawn as Zanoprima is aware that the level of R-isomer in Hangsen MOTiVO™ branded Synthetic S-Nicotine is 0.1-0.15%, which is consistent with the claims made for the R-isomer in CN112795603A.

3.4 Either way, Zanoprima believes that the production of Hangsen MOTiVO™ Synthetic S-Nicotine is an unauthorised use of Zanoprima's patented process, and Hangsen is importing, selling, and offering Hangsen MOTiVO™ Synthetic S-Nicotine in the US and UK, including through the Website, the Genmist Shop (available at https://www.genmist.shop/) and third party importers. Hangsen's conduct in doing this

is a clear violation of s60(1)(C) of the Patents Act 1977(UK) and a clear violation of 35 U.S.C. § 271 of the U.S. Code (US).

4. Next steps

Hangsen's infringements of the Zanoprima Patents are unacceptable. In the circumstances, Zanoprima requires Hangsen to provide a response to the matters raised in this letter and to return signed undertakings in the form attached by no later than **4 pm GMT** on **7 January 2022**. Should Hangsen fail to provide an adequate response and the requested undertakings by such time, Zanoprima reserves the right to issue proceedings without further notice.

Please note that if proceedings do become necessary, the remedies available to our client are broad, and include injunctive relief, damages, and delivery up or destruction of all infringing items.

Our client's rights are reserved.

Yours faithfully,

Norton Rose Fulbright LLP

Norton Rose Fulbright LLP

NORTON ROSE FULBRIGHT

Schedule 1: Undertakings to be provided by Hangsen

[ON HEADED NOTEPAPER OF HANGSEN]

Zanoprima Lifesciences Limited
C/O Norton Rose Fulbright
3 More London Riverside
London SE1 2AQ
United Kingdom

[DATE]

Dear Zanoprima Lifesciences Limited (**Zanoprima**)

In consideration of Zanoprima refraining from bringing legal proceedings against Hangsen International Group Ltd, either in its own right or through its subsidiaries and associated group entities (**Hangsen**) for infringing Zanoprima's rights under European patent number 3653617 and US patent number 10913962 (the Zanoprima Patents) by virtue of Hangsen importing or disposing of, or offering to dispose of, Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ branded Synthetic S-Nicotine in the US and UK, Hangsen undertakes, whether acting by itself, its directors, officers, employees, subsidiaries or associated entities or any of them howsoever as follows:

1. To immediately cease importing, disposing of and/or offering to dispose of in the US and UK, Hangsen MOTiVO™ Synthetic S-Nicotine products, products that use MOTiVO™ Synthetic S-Nicotine and any other products which would amount to an infringement of the Zanoprima Patents.

2. Not now or any time in the future, make, import or dispose of (or offer to dispose of) Hangsen MOTiVO™ Synthetic S-Nicotine products, products that use MOTiVO™ Synthetic S-Nicotine or any other products in the UK and US which would amount to an infringement of the Zanoprima Patents.

3. To deliver up to Zanoprima Lifesciences (C/O Norton Rose Fulbright LLP at 3 More London Riverside, London, United Kingdom SE1 2AQ) by **21 January 2022**:

   (a) all of the Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine in Hangsen's possession, custody or control in the UK; and

   (b) all documents in its possession, custody or control which relate to the manufacture, importation and/or disposal or purported disposal of any Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine in the UK.

4. To deliver up to Zanoprima Lifesciences (C/O Norton Rose Fulbright at 1301 McKinney, Suite 5100, Houston, Texas, 77010-3095, United States) by **21 January 2022**:

   (c) all of the Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine in Hangsen's possession, custody or control in the US; and

   (d) all documents in its possession, custody or control which relate to the manufacture, importation and/or disposal or purported disposal of any Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine in the US.

5. By **21 January 2022** to retrieve all stocks of Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine from all purchasers in the UK and US to the extent that such stocks remain within Hangsen's power, and to use Hangsen's best endeavours to retrieve such goods which are no longer within its power and deliver up these stocks to Zanoprima in accordance with paragraphs 3 and 4 above.

6. To pay such damages or profits, at Zanoprima's discretion, relating to Hangsen's importation, disposal and/or purported disposal of Hangsen MOTiVO™ Synthetic S-Nicotine products and products that use MOTiVO™ Synthetic S-Nicotine, as may be agreed or, in default of agreement, determined by the court.

7. By **28 January 2022**, provide Zanoprima with a sworn affidavit verifying that the obligations imposed by paragraphs 1-6 have been fully complied with.

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A duly authorised signatory for and on behalf of Hangsen