

ANSWER: Thorne admits ChromaDex purports to bring this counterclaim concerning certain Thorne dietary-supplement formulations and U.S. Patent No. 12,252,506 (the “’506 Patent”). Thorne admits that the parties’ earlier litigation involved different patents related to NR. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 1.

2. The ’506 Patent belongs to a patent family from The Queen’s University of Belfast (“QUB”) distinct from the Dartmouth patent family involved in the parties’ earlier litigation. It names Marie Migaud, Philip Redpath, Kerri Crossey, and Mark Doherty as inventors. Its specification describes limitations that made known NR-preparation protocols ill-suited to commercial or industrial scale and approaches permitting selection of a desired counterion while reducing processing burdens. The asserted claims define the claimed subject matter through specified chemical structures, categories of counterions, and other limitations.

ANSWER: Thorne admits that Marie Migaud, Philip Redpath, Kerri Crossey, and Mark Doherty are named as inventors on the face of the ’506 Patent and that QUB is the assignee on the face of the patent. Thorne admits that the parties’ earlier litigation was not directed to the ’506 Patent. Thorne admits that the title of the ’506 Patent is “Methods of Preparing Nicotinamide Riboside and Derivatives Thereof.” To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 2.

3. QUB solely owned the ’506 Patent family and, by November 23, 2015, had exclusively licensed it to ChromaDex. The parties’ agreements assigned ChromaDex responsibility for commercializing, prosecuting, maintaining, and enforcing the licensed family. Effective December 16, 2025, QUB assigned its scheduled rights in the ’506 Patent and associated pre- and post-assignment enforcement claims to ChromaDex, subject to QUB’s retained nonexclusive right to use the assigned patents for internal, noncommercial academic purposes.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 3, and, therefore, denies them.

4. Drawing on foundational discoveries by university scientists, ChromaDex built a commercial platform around NR products, research, and supply. ChromaDex developed and managed manufacturing and supply relationships, established product specifications and quality controls, pursued regulatory pathways, supported scientific and clinical research, and developed commercial customers and markets. In 2013, ChromaDex

commercialized NIAGEN[®], its proprietary form of NR chloride, as a food-grade ingredient. NR chloride is a different salt from the NR hydrogen malate ingredient identified on the labels of Thorne's accused formulations.

ANSWER: On information and belief, Thorne admits that NIAGEN[®] is NR chloride, and that NR chloride is a different salt from NR hydrogen malate. Thorne admits that it markets certain products that contain NR hydrogen malate ("Thorne NR Products"), including ResveraCel[®], NiaCel[®] 400, Collagen Plus, Collagen Fit, SynaQuell+[™], Brain Factors, Daily Greens Plus. Thorne lacks knowledge or information sufficient to form a belief as to the truth of the remaining allegations of Paragraph 4, and, therefore, denies them.

5. Thorne was an early commercial participant in the NIAGEN[®] launch. On July 9, 2013, Thorne entered a written agreement with ChromaDex to purchase NIAGEN[®] for use in dietary supplements. The agreement described NIAGEN[®] as a "novel and proprietary" ingredient and contemplated product-launch, commercial, and research activities. Thorne later acknowledged that it had obtained NIAGEN[®] under supply agreements beginning in July 2013. Thorne ended the operative supply relationship effective December 31, 2020.

ANSWER: Thorne admits that it entered into a supply agreement with ChromaDex in 2013 to purchase NR chloride and that the supply relationship ended. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 5.

6. After that relationship ended, Thorne marketed products labeled as containing NR hydrogen malate, a different NR salt. For purposes of this counterclaim, the "Accused Products" are ResveraCel[®], NiaCel[®] 400, Collagen Plus, Collagen Fit, SynaQuell[™], SynaQuell+[™], Advanced Nutrients, Brain Factors, and Daily Greens Plus, only insofar as those products are or were formulated with nicotinamide riboside hydrogen malate, and only with respect to the infringing acts occurring on or after the issuance of the '506 Patent. The accused Advanced Nutrients formulation is limited to the legacy SKU VMX 240-capsule formulation labeled as containing 45 mg NRHM per eight-capsule serving. The accused SynaQuell formulations include the SKU DID003 SynaQuell formulation labeled as containing 207.5 mg NRHM per two-scoop serving and SKU DID004 SynaQuell+ formulation labeled as containing 415 mg NRHM per scoop.

ANSWER: Thorne admits that it markets the Thorne NR Products. Thorne admits that ChromaDex purports to define the "Accused Products" as set forth in Paragraph 6. Thorne denies that any of the "Accused Products" infringe the '506 Patent. To the extent not expressly admitted

herein, Thorne denies the remaining allegations of paragraph 6.

7. Publicly available current and historical labels and product listings identify the Accused Products as containing NR hydrogen malate. Based on those labels and the chemical identity of the labeled ingredient, ChromaDex alleges on information and belief that each Accused Product meets every limitation of one or more claims asserted below. The earlier Dartmouth proceedings neither adjudicated nor otherwise involved any claim of the '506 Patent. This case turns on the '506 Patent's own claim language and the compositions of the Accused Products.

ANSWER: Thorne admits it markets the Thorne NR Products. Thorne admits that the parties' earlier litigation was not directed to the '506 Patent. Thorne denies that any of the "Accused Products" infringe the '506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 7.

8. After the '506 Patent issued, ChromaDex gave Thorne written notice of the patent and ChromaDex's infringement position. ChromaDex's July 22, 2025 correspondence explained, among other things, that claims 1 and 26 read on NR malate, discussed dependent claims 2 and 3, and stated that Thorne's sale of NiaCel and any other Thorne product containing NR malate infringed the '506 Patent. Counterclaim Ex. 1. ChromaDex invited an amicable resolution. Amazon's later APEX notice included listings for NiaCel 400, SynaQuell, and ResveraCel. As alleged below, after receiving actual notice, Thorne continued its accused offers to sell and sales. ChromaDex alleges that Thorne's post-notice infringement has been intentional, knowing, and willful.

ANSWER: Thorne admits ChromaDex sent Thorne a letter dated July 22, 2025. Thorne admits that ChromaDex initiated an APEX evaluation regarding Thorne's products. Thorne denies that it has infringed the '506 Patent, that any such infringement was intentional, knowing, or willful. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 8.

Nature of the Case

9. ChromaDex brings this counterclaim under 35 U.S.C. § 271 for Thorne's infringement of claims 1-3, 10-12, and 26 ("Asserted Claims") of U.S. Patent No. 12,252,506 ("'506 Patent") through its own offers to sell and sales of the Accused Products in the United States, including infringement committed after Thorne received actual notice of the '506 Patent and ChromaDex's infringement position.

ANSWER: Thorne admits that ChromaDex purports to bring this counterclaim under 35 U.S.C.

§ 271 for alleged infringement of claims 1-3, 10-12, and 26 of the '506 Patent. Thorne denies that it has infringed any claim of the '506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 9.

The Parties

10. Counterclaim-Plaintiff ChromaDex, Inc. is a California corporation with its principal place of business at 10900 Wilshire Boulevard, Suite 600, Los Angeles, California 90024. ChromaDex is a wholly owned subsidiary of Niagen Bioscience, Inc., formerly known as ChromaDex Corporation.

ANSWER: On information and belief, admitted.

11. In December 2025, ChromaDex and QUB executed a written agreement entitled "Assignment," effective December 16, 2025 (the "QUB Assignment Agreement"). Under the QUB Assignment Agreement, QUB assigned and transferred to ChromaDex all of QUB's worldwide rights, title, and interest in the patents identified therein, including the '506 Patent, together with all rights to bring or defend infringement claims and retain damages recovered for infringement occurring before, on, or after December 16, 2025. The QUB Assignment Agreement reserves for QUB, its employees, and its students a nonexclusive, irrevocable, worldwide, royalty-free right to use the assigned patents for internal, noncommercial research, publication, and teaching. Accordingly, ChromaDex owns the '506 Patent and possesses the right to enforce it and recover damages for infringement. A true and correct copy of the publicly-filed QUB Assignment Agreement is attached hereto as Counterclaim Exhibit 3.

ANSWER: Thorne admits that a heavily redacted document purporting to be the QUB Assignment Agreement is attached as Counterclaim Exhibit 3. Thorne lacks knowledge or information sufficient to form a belief as to the truth of the remaining allegations of Paragraph 11, and, therefore, denies them.

12. Counterclaim-Defendant Thorne Research, Inc. is a South Carolina corporation with its principal place of business at 620 Omni Industrial Boulevard, Summerville, South Carolina 29486.

ANSWER: Admitted.

13. Counterclaim-Defendant Thorne HealthTech is a Delaware corporation with its principal place of business at 620 Omni Industrial Blvd., Summerville, South Carolina 29486. Thorne Research is a wholly owned subsidiary of Thorne HealthTech.

ANSWER: Admitted.

14. On information and belief, Thorne has marketed, offered for sale, and sold the Accused Products in and from South Carolina and directly to consumers in the United States, including through Thorne's website and, at least as an additional channel, through Amazon in connection with its relationship with Pattern. *See* Compl. ¶¶ 34, 36, 40-41; Counterclaim Exs. 4-23. ChromaDex asserts this counterclaim against Thorne based on those offers to sell and sales.

ANSWER: Thorne admits that it sells certain Thorne NR Products in and from South Carolina and to consumers in the United States, including through its website. Thorne admits that it has a contractual relationship with Pattern relating to the sale of Thorne NR Products on Amazon. Thorne denies that any such activities constitute infringement of the '506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 14.

Jurisdiction and Venue

15. ChromaDex repeats and incorporates by reference the foregoing allegations as though fully set forth herein.

ANSWER: Thorne incorporates by reference its responses to all foregoing paragraphs as if set forth fully herein.

16. This counterclaim arises under the patent laws of the United States, including 35 U.S.C. § 271 *et seq.* This Court has subject matter jurisdiction over this counterclaim under 28 U.S.C. §§ 1331 and 1338(a).

ANSWER: Thorne admits that this Court has subject matter jurisdiction over this matter. Except as expressly admitted herein, the allegations of paragraph 16 are denied.

17. This Court has personal jurisdiction over Thorne because Thorne initiated this action and invoked this Court's jurisdiction to adjudicate a controversy concerning the same patent, Accused Products, and alleged acts addressed by this counterclaim. Thorne also conducts substantial business in this District.

ANSWER: Thorne does not contest personal jurisdiction for the limited purpose of this civil action only. Except as expressly admitted herein, the allegations of paragraph 17 are denied.

18. Venue is proper because Thorne selected this District to adjudicate this patent dispute and

ChromaDex's counterclaim arises from the same patent, products, and controversy. Venue is also independently proper as to Thorne Research under 28 U.S.C. § 1400(b) because Thorne Research is incorporated in South Carolina and therefore resides in this District for patent-venue purposes.

ANSWER: Thorne does not contest venue for the limited purpose of this civil action only. Except as expressly admitted herein, the allegations of paragraph 18 are denied.

Factual Background

19. The '506 Patent, entitled "Methods of Preparing Nicotinamide Riboside and Derivatives Thereof," names Marie Migaud, Philip Redpath, Kerri Crossey, and Mark Doherty as inventors. It issued on March 18, 2025, and claims priority through a continuation chain to a July 29, 2013, United Kingdom application.

ANSWER: Thorne admits "Methods of Preparing Nicotinamide Riboside and Derivatives Thereof" is the title on the face of the '506 Patent; Marie Migaud, Philip Redpath, Kerri Crossey, and Mark Doherty are named as inventors on the face of the '506 Patent; March 18, 2025 is the issue date listed on the face of the '506 Patent; and a United Kingdom application from July 29, 2013 appears on the face of the '506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 19.

20. The specification describes practical problems with then-known protocols for preparing NR compounds. According to the patent, those protocols could yield a triflate salt unsuitable for nutritional use and require added ion-exchange and chromatographic processing. The patent also describes NR's chemical lability and an exothermic reaction as creating purity and scale-up concerns. '506 Patent at 1:42–2:9.

ANSWER: Thorne admits the '506 Patent states, on its face, "known synthetic protocols for preparing nicotinamide riboside and derivatives thereof have disadvantages, rendering them unsuitable for scaling up for commercial or industrial use." *Id.* at 1:45–48. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 20.

21. Against that background, the specification describes approaches intended to provide an efficient preparation method and permit introduction of a selected counterion. *Id.* at 2:18–35. It further explains that the disclosed work can reduce solvent use, purification, and reaction time while producing the desired beta anomer. *Id.* at 10:36–67.

ANSWER: Thorne admits the '506 Patent states, on its face, “an object of the invention to provide a method for the preparation of nicotinamide riboside and derivatives thereof, whereby the method may be used to introduce a counter ion of choice to the prepared compounds[.]” *Id.* at 2:29-32. Thorne admits that the '506 Patent purports to state “[a]dvantages of the invention” in the specification. *Id.* at 10:35. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 21.

22. The issued patent includes method claims and claims directed to defined compounds and nutritional-supplement compositions. ChromaDex asserts claims 1-3, 10-12, and 26 of the '506 Patent. The Asserted Claims define the accused subject matter through specified structural features, categories of counterions, and additional limitations.

ANSWER: Paragraph 22 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits the '506 Patent includes method and composition claims, and ChromaDex asserts claims 1-3, 10-12, and 26 of the '506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 22.

23. ChromaDex and QUB maintained a broader research relationship documented in an October 9, 2015 Joint Ownership and Management Agreement. That agreement addressed certain jointly owned inventions and patent applications. The '506 Patent family was different: QUB solely owned it and exclusively licensed it to ChromaDex under a separate License Agreement dated November 23, 2015.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 23, and, therefore, denies them.

24. The QUB Assignment Agreement recites that QUB granted ChromaDex an exclusive license to commercialize the covered patents and that ChromaDex was responsible for prosecuting, maintaining, and enforcing them. ChromaDex therefore held exclusive commercial and enforcement rights in the '506 Patent family before the patent issued and this dispute arose.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 24, and, therefore, denies them.

25. Effective December 16, 2025, under the QUB Assignment Agreement, QUB assigned to ChromaDex its worldwide right, title, and interest in the patents identified in the Assignment, including the '506 Patent. The QUB Assignment Agreement expressly transferred associated claims and causes of action arising from ownership, together with the right to retain resulting damages, for conduct occurring before, on, or after the date.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 25, and, therefore, denies them.

26. QUB reserved a nonexclusive, irrevocable, worldwide, royalty-free right for QUB, its employees, and its students to use the assigned patents for internal, noncommercial research, publication, and teaching. Subject to that reservation, ChromaDex is the current owner of the '506 Patent and possesses the right to enforce it and seek relief for infringement occurring before and after December 16, 2025.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 26, and, therefore, denies them.

27. NR is a form of vitamin B3 and a precursor to NAD⁺, a coenzyme involved in cellular metabolism. Academic scientists, including Dr. Charles Brenner, made foundational discoveries that generated modern scientific interest in NR. ChromaDex built on that work by licensing defined university technologies, conducting NR-related research and development through its own scientists, and undertaking the development needed to translate laboratory science into reliable commercial products. Those licensed technologies belong to patent families distinct from the QUB-owned '506 family.

ANSWER: Thorne admits that NR is a form of vitamin B3 and a precursor to NAD⁺. Thorne lacks knowledge or information sufficient to form a belief as to the truth of the remaining allegations of Paragraph 27, and, therefore, denies them.

28. ChromaDex developed and managed commercial manufacturing and supply relationships, established product specifications and quality controls, performed and supported safety, toxicology, and clinical work, pursued applicable regulatory pathways, and developed commercial customers. Those activities supplied scientific, technical, and commercial infrastructure for consistently supplying NR ingredients at consumer-product scale.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 28, and, therefore, denies them.

29. In 2013, ChromaDex commercialized food-grade NIAGEN[®], its proprietary nicotinamide riboside chloride ingredient. NIAGEN[®] is NR chloride. ChromaDex supplies NIAGEN[®]

to customers that formulate dietary supplements, while Niagen Bioscience and its consolidated subsidiaries market and sell finished TRU NIAGEN[®] oral dietary supplements containing NIAGEN[®].

ANSWER: Thorne admits that ChromaDex has sold NR chloride under the tradename NIAGEN[®].

Thorne lacks knowledge or information sufficient to form a belief as to the truth of the remaining allegations of Paragraph 29, and, therefore, denies them.

30. ChromaDex submitted New Dietary Ingredient notifications for NIAGEN[®] to the U.S. Food and Drug Administration (“FDA”) in 2015 and 2017. Separately, an independent expert panel concluded that specified uses of NIAGEN[®] were generally recognized as safe (“GRAS”), and ChromaDex submitted a notice concerning that GRAS conclusion to FDA in 2016. FDA responded that it had no questions at that time regarding ChromaDex’s GRAS conclusion, while expressly stating that the agency had not made its own GRAS determination.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 30, and, therefore, denies them.

31. ChromaDex has also conducted NR research through its own scientists and supported independent investigators by supplying characterized materials and technical support. Its patent, licensing, manufacturing, regulatory, research, and supply activities form parts of the operating platform through which it has made NR products and defined NR technologies commercially available.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 31, and, therefore, denies them.

32. Thorne identifies the Accused Products as dietary supplements. Publicly available current and historical labels identify the following amounts of nicotinamide riboside hydrogen malate: 415 milligrams per two-capsule serving of ResveraCel[®]; 415 milligrams per NiaCel[®] 400 capsule; 125 milligrams per Collagen Plus scoop; 125 milligrams per Collagen Fit scoop; 207.5 milligrams per two-scoop serving of SynaQuell[™]; 415 milligrams per SynaQuell+[™] scoop; 45 milligrams per eight-capsule serving of the accused Advanced Nutrients formulation; 415 milligrams per Brain Factors capsule; and 100 milligrams per Daily Greens Plus scoop. Counterclaim Exs. 4-24.

ANSWER: Thorne admits the amounts of NR hydrogen malate as set forth in Paragraph 32 appear on the face of the referenced Counterclaim Exhibits. To the extent not admitted, Thorne denies the remaining allegations of Paragraph 32.

33. Publicly available Thorne product pages offer or have offered the Accused Products for purchase, including after the '506 Patent issued. Counterclaim Exs. 4-23 Those materials include current listings for ResveraCel[®], NiaCel[®] 400, Collagen Plus, Collagen Fit, SynaQuell[™], SynaQuell+[™], Brain Factors, and Daily Greens Plus for direct purchase. Counterclaim Exs. 3-17 and 19-23. An archived Thorne product page, dated November 13, 2025, offered the accused Advanced Nutrients formulation for direct purchase. Counterclaim Ex. 18. On information and belief, Amazon identifies current listings for the Accused Products under the following ASINs: B097Z5387H (ResveraCel[®]), B08WPXPTD8 (NiaCel[®] 400), B09BT31TW2 (Collagen Plus), B0B7K9Y1FD (Collagen Fit), B0B14F9PSR (SynaQuell[™]), B0B148MZ9G (SynaQuell+[™]), B0B142CMJB (Brain Factors), and B0B7PXPY5Q (Daily Greens Plus). On information and belief, an Amazon listing under B000FGWCHI offered the Accused Advanced Nutrients formulation. ChromaDex's allegations concerning Advanced Nutrients are limited to the post-issuance formulation containing NR hydrogen malate. ChromaDex does not accuse any current or historical Advanced Nutrients formulation that omits NR hydrogen malate.

ANSWER: Thorne admits that it has offered certain Thorne NR Products through its website and that certain Thorne NR Products are offered through Amazon. Thorne admits that the referenced Counterclaim Exhibits purport to reflect product pages. To the extent not admitted, Thorne denies the remaining allegations of Paragraph 33.

34. Nicotinamide riboside hydrogen malate identifies an NR cation paired with a hydrogen-malate counterion. Hydrogen malate is the monoanionic form of malic acid, a hydroxy-substituted dicarboxylic acid. Based on the public labels and the chemical identity of the labeled ingredient, ChromaDex alleges that each Accused Product meets the limitations of the Asserted Claims as set forth more fully in the claim chart attached as Counterclaim Exhibit 24.

ANSWER: Paragraph 34 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits nicotinamide riboside hydrogen malate is an NR cation paired with a hydrogen-malate counterion, and that hydrogen malate is the monoanionic form of malic acid. Thorne denies that any Accused Product meets the limitations of any Asserted Claim of the '506 Patent. To the extent not admitted, Thorne denies the remaining allegations of Paragraph 34.

35. Thorne's Complaint alleges that Thorne manufactures and sells identified NR products from South Carolina facilities, sells products directly through thorne.com, maintains a contractual relationship with Pattern concerning Amazon sales, and sells other products

containing NR that were not identified in the APEX notice. Compl. ¶¶ 34, 36, 40–41. The Complaint collectively defines Thorne products containing NR malate as the “Thorne NR Products.” Thorne’s Inter Partes Review (“IPR”) petition likewise identifies both Counterclaim-Defendants as petitioners and real parties-in-interest and states collectively that Thorne purchases NR products and maintains the Pattern relationship. IPR2026-00400, Petition at 62.

ANSWER: Thorne states that the Complaint and the IPR petition, IPR2026-00400, are documents that speak for themselves. To the extent that the allegations in Paragraph 35 characterize or summarize the Complaint or the IPR petition in a manner inconsistent with those documents, Thorne denies those allegations. To the extent not admitted, Thorne denies the remaining allegations of Paragraph 35.

36. Based on Thorne’s own allegations and representations and the public product records described above, ChromaDex alleges on information and belief that Thorne offered for sale each Accused Product in the United States after the ’506 Patent issued and sold one or more Accused Products.

ANSWER: Thorne incorporates by reference its responses to all foregoing paragraphs. Paragraph 36 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne denies the allegations.

37. On May 13, 2025, less than two months after the ’506 Patent issued, ChromaDex sent Thorne Research a letter notifying it of the patent’s issuance, stating that ChromaDex held exclusive rights under the patent, requesting information concerning the process used to manufacture Thorne’s NR-malate ingredient, and inviting the parties to discuss potential cooperation.

ANSWER: Paragraph 37 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits that ChromaDex sent Thorne Research a letter dated May 13, 2025 that made reference to the issuance of the ’506 Patent. Thorne admits the letter requested information concerning Thorne’s manufacturing process and invited discussion, but denies ChromaDex’s characterization of the letter’s purpose and legal effect. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 37.

38. Thorne Research responded on July 17, 2025 and requested further explanation. On July 22, 2025, ChromaDex provided a claim- and product-specific explanation of its infringement position. ChromaDex identified claims 1 and 26, discussed dependent claims 2 and 3, reproduced claim 1, and explained why it understood claims 1 and 26 to read on NR malate.

ANSWER: Paragraph 38 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits that Thorne Research responded to ChromaDex on July 17, 2025 explaining that the May 13, 2025 correspondence contained no context for or explanation of the purported notice regarding the '506 Patent. Thorne admits that ChromaDex sent a follow-up letter on July 22, 2025. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 38.

39. ChromaDex identified NiaCel as a Thorne product containing NR malate and stated its position that sales of NiaCel and any other Thorne product containing NR malate infringed the '506 Patent. ChromaDex reiterated its preference for an amicable resolution and, if Thorne declined, asked it to cease the accused sales and destroy existing NR-malate inventory in its possession or control. ChromaDex reserved all rights and remedies.

ANSWER: Paragraph 39 contains legal conclusions to which no answer is required. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 39.

40. Niagen Bioscience's Form 10-K for fiscal year 2025 lists the '506 Patent, uses the consolidated description "Owned by Niagen Bioscience," and defines the reporting company to include Niagen Bioscience and its wholly owned subsidiaries. Thorne cited and relied on that disclosure in its Complaint. Compl. ¶ 22 & Ex. 5 at 9-10. Thorne's reliance on the disclosure is further evidence that Thorne had actual knowledge of the '506 Patent at the time the 10-K was filed and at least by no later than April 28, 2026.

ANSWER: Paragraph 40 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits it cited Niagen Bioscience's Form 10-K for fiscal year 2025 in the Complaint. That document speaks for itself. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 40.

41. On April 10, 2026, Amazon transmitted an APEX notice identifying the '506 Patent and three listings, including ResveraCel, NiaCel 400 and SynaQuell. Thorne filed this action eighteen days later and alleged an actual controversy concerning whether its broadly

defined “Thorne NR Products” infringe the ’506 Patent. On information and belief, Amazon briefly removed one or more of the identified listings in connection with the APEX process and later restored them.

ANSWER: Paragraph 41 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits that, as detailed in the Complaint, Pattern received notice that ChromaDex initiated an APEX evaluation regarding Thorne’s products and that Pattern sent that notice to Thorne. Thorne admits that the notice stated that ChromaDex claimed that ten ASINs sold on Amazon infringed the ’506 Patent and that three of the ASINs were associated with Thorne products: ResveraCel[®], NiaCel[®] 400 and SynaQuell[™]. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 41.

42. Thorne had actual knowledge of the ’506 Patent no later than May 13, 2025 and, since at least July 22, 2025, had notice of ChromaDex’s specific position concerning NiaCel and other Thorne products containing NR malate. On information and belief, Thorne continued the accused offers for sale and sales after receiving that notice. That post-notice conduct included continued offers and sales of ResveraCel[®], NiaCel[®] 400, Collagen Plus, Collagen Fit, SynaQuell[™], SynaQuell+[™], Brain Factors, and Daily Greens Plus and, on information and belief, offers and sales of the accused legacy Advanced Nutrients formulation through at least May 2026.

ANSWER: Paragraph 42 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits it received ChromaDex’s correspondence dated May 13, 2025 and July 22, 2025. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 42.

43. Thorne does not have a license or other authority from ChromaDex to practice the Asserted Claims of the ’506 Patent. Those unauthorized offers to sell and sales invade ChromaDex’s exclusive patent rights and compete in the commercial market for NR supplements.

ANSWER: Paragraph 43 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits it does not have an express license to the ’506 Patent. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 43.

44. Thorne’s infringement has caused and will continue to cause ChromaDex damages in an

amount to be determined at trial, including lost profits and no less than a reasonable royalty. Continued infringement also causes ongoing harm for which ChromaDex may obtain injunctive relief upon proof of the governing equitable requirements.

ANSWER: Denied.

Count I
(Direct Infringement of U.S. Pat. No. 12,252,506)

45. ChromaDex repeats and incorporates by reference the foregoing allegations as though fully set forth herein.

ANSWER: Thorne reasserts and incorporates by reference its answers to paragraphs 1-44 as if fully set forth herein.

46. ChromaDex owns the '506 Patent under the QUB Assignment Agreement and possesses the right to enforce it and recover damages for infringement occurring before, on, or after December 16, 2025 pursuant to the transferred claims and causes of action. QUB's reserved nonexclusive internal academic-use right does not include commercial ownership of the '506 Patent.

ANSWER: Thorne lacks knowledge or information sufficient to form a belief as to the truth of the allegations of Paragraph 46, and, therefore, denies them.

47. The United States Patent and Trademark Office duly and legally issued the '506 Patent on March 18, 2025. A true and correct copy of the '506 Patent is attached to the Complaint as Exhibit 1.

ANSWER: Paragraph 47 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits that the '506 Patent states on its face that it issued on March 18, 2025, and that a copy of the '506 Patent is attached to the Complaint as Exhibit 1. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 47.

48. On information and belief, Thorne has offered to sell and sold each Accused Product in the United States after the '506 Patent issued. Thorne continues to offer to sell and sell ResveraCel[®], NiaCel[®] 400, Collagen Plus, Collagen Fit, SynaQuell[™], SynaQuell+[™], Brain Factors, and Daily Greens Plus. With respect to Advanced Nutrients, on information and belief, Thorne offered to sell and sold the accused NR-hydrogen-malate formulation during a post-issuance period extending through at least May 2026.

ANSWER: Paragraph 48 contains legal conclusions to which no answer is required. To the extent

an answer is required, Thorne admits that it has offered to sell and sold certain Thorne NR Products in the United States. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 48.

49. ChromaDex alleges that each Accused Product meets the limitations of the Asserted Claims as set forth more fully in the claim chart. Counterclaim Ex. 24.

ANSWER: Denied.

50. On information and belief, each Accused Product's labeled nicotinamide riboside hydrogen malate ingredient meets claim 1. Counterclaim Ex. 24. Specifically:

- a) Each Accused Product's label identifies its active NR ingredient as nicotinamide riboside hydrogen malate, a compound having the general structure of Formula IV or the beta anomer thereof.
- b) For the labeled NR-hydrogen-malate compound, n is 0 and Y is absent.
- c) Within the Formula IV NR moiety, m is 1.
- d) R1 is an unsubstituted primary amino group, NH₂, and R2, R3, R4, and R5 are each hydrogen.
- e) R6, R7, and R8 are each hydrogen.
- f) X is the hydrogen-malate counterion. Hydrogen malate is the monoanion of malic acid, a hydroxy-substituted dicarboxylic acid, and therefore is an anion of a substituted carboxylic acid within claim 1.
- g) For those reasons, each Accused Product's labeled ingredient meets every limitation of claim 1.

ANSWER: Denied.

51. Each Accused Product meets claim 2 because R1 is NH₂. Counterclaim Ex. 24.

ANSWER: Denied.

52. Each Accused Product meets claim 3 because R6, R7, and R8 are each hydrogen, which claim 3 expressly permits. Counterclaim Ex. 24.

ANSWER: Denied.

53. On information and belief, the nicotinamide riboside hydrogen malate identified on each

Accused Product's label has the unprotected beta-D structure depicted in claim 26, which depends from claim 1. As detailed in the attached claim chart, that compound satisfies the depicted structural limitations, and its X- counterion is hydrogen malate—the monoanion of malic acid, a hydroxy-substituted dicarboxylic acid. Counterclaim Ex. 24. ChromaDex therefore alleges that each Accused Product contains a compound meeting every limitation of claim 26.

ANSWER: Denied.

54. On information and belief, each Accused Product meets claim 10. Counterclaim Ex. 24. Specifically:

- a) Each Accused Product is or was labeled and offered as a dietary supplement and therefore is a nutritional-supplement composition.
- b) The labels identify the following amounts of nicotinamide riboside hydrogen malate: 415 milligrams per two-capsule serving of ResveraCel®; 415 milligrams per NiaCel® 400 capsule; 125 milligrams per Collagen Plus scoop; 125 milligrams per Collagen Fit scoop; 207.5 milligrams per two-scoop serving of SynaQuell™; 415 milligrams per SynaQuell+™ scoop; 45 milligrams per eight-capsule serving of the accused Advanced Nutrients formulation; 415 milligrams per Brain Factors capsule; and 100 milligrams per Daily Greens Plus scoop. Each is the compound alleged above to meet claim 1.

ANSWER: Denied.

55. Each Accused Product meets claim 11 because it meets claim 10, R1 is NH₂, and hydrogen malate is an anion of a hydroxy-substituted dicarboxylic acid. Counterclaim Ex. 24.

ANSWER: Denied.

56. Each Accused Product meets claim 12 because it meets claim 10, R1 is NH₂, and R6, R7, and R8 are each hydrogen, which claim 12 expressly permits. Counterclaim Ex. 24.

ANSWER: Denied.

57. Thorne has no license or other authority from ChromaDex to practice the Asserted Claims of the '506 Patent. By offering to sell and selling the Accused Products in the United States, Thorne has directly and literally infringed under 35 U.S.C. § 271(a). Thorne continues to infringe through its offers to sell and sales of ResveraCel®, NiaCel® 400, Collagen Plus, Collagen Fit, SynaQuell™, SynaQuell+™, Brain Factors, and Daily Greens Plus.

ANSWER: Denied.

58. Thorne had actual knowledge of the '506 Patent no later than May 13, 2025. Since at least July 22, 2025, Thorne has had actual notice of ChromaDex's specific charge that Thorne's

sale of NiaCel and any other Thorne product containing NR malate infringed at least claims 1 and 26 of the '506 Patent. On information and belief, Thorne continued its accused offers for sale and sales after receiving that notice.

ANSWER: Paragraph 58 contains legal conclusions to which no answer is required. To the extent an answer is required, Thorne admits it received ChromaDex's correspondence dated May 13, 2025 and July 22, 2025. To the extent not expressly admitted herein, Thorne denies the remaining allegations of paragraph 58.

59. On information and belief, Thorne's infringement of the '506 Patent after July 22, 2025, has been and continues to be willful, intentional, and deliberate. Despite its actual knowledge of the '506 Patent, ChromaDex's detailed infringement position, and the NR-hydrogen-malate composition identified on the Accused Products' labels, Thorne knowingly and deliberately continued the infringing offers to sale and sales alleged above.

ANSWER: Denied.

60. Thorne's infringement has caused and will continue to cause ChromaDex damages in an amount to be proved at trial, but no less than a reasonable royalty from the date permitted by applicable law, together with interest and costs under 35 U.S.C. § 284. ChromaDex further seeks enhanced damages under 35 U.S.C. § 284 on Thorne's willful infringement.

ANSWER: Denied.

61. Unless enjoined after proof of the governing equitable requirements, Thorne's continuing infringement will continue to impair ChromaDex's exclusive rights under the '506 Patent.

ANSWER: Denied.

Thorne denies all allegations, including all subparts A-G, in the Relief Requested by Defendants in the Answer to Plaintiffs' Complaint and Counterclaim.

AFFIRMATIVE DEFENSES

Thorne alleges and asserts the following defenses in further response to the Counterclaim, undertaking the burden of proof only as to those defenses deemed affirmative defenses by law, regardless of how such defenses are denominated herein. In addition to the affirmative defenses asserted below, Thorne reserves the right to assert additional defenses, affirmative or otherwise, during the course of discovery.

FIRST AFFIRMATIVE DEFENSE**(Failure to State a Claim)**

The Counterclaim fails to state a claim upon which relief may be granted. In particular, the counterclaim fails to state how and where each limitation of any valid claim of the '506 Patent is found in any Thorne NR Products.

SECOND AFFIRMATIVE DEFENSE**(Non-Infringement of the '506 Patent)**

The manufacture, sale, or offer for sale of any Thorne NR Products do not and would not infringe, directly or indirectly, either literally or under the doctrine of equivalents, any valid or enforceable claim of the '506 Patent.

THIRD AFFIRMATIVE DEFENSE**(Invalidity of the '506 Patent)**

The asserted claims of the '506 Patent are invalid at least due to anticipation by or obviousness over the prior art, because they are directed towards ineligible subject matter, and for failing to comply with the requirements for patentability as set forth in Title 35, United States Code, including but not limited to, 35 U.S.C. §§ 101, 102, 103, and/or 112 thereof, and the rules, regulations, and laws pertaining thereto, and/or obviousness type double patenting.

FOURTH AFFIRMATIVE DEFENSE**(Prosecution History Estoppel)**

The Counterclaim of patent infringement is barred by the doctrine of prosecution history estoppel to the extent ChromaDex's interpretation of the claims of the '506 Patent extends beyond or is inconsistent with statements, amendments, or positions made during prosecution. ChromaDex is further estopped from construing one or more claims of the '506 Patent to claim

scope ceded during the prosecution of the '506 Patent and/or related patent(s) to, for example, avoid prior art.

FIFTH AFFIRMATIVE DEFENSE

(Limitation on Damages)

Any potential recovery by ChromaDex is limited to any infringement committed after Thorne received actual notice of the '506 Patent and ChromaDex's infringement positions. Further, any potential recovery by ChromaDex is limited to any infringement committed since the valid issuance of the '506 Patent. Further, any potential recovery by ChromaDex is limited by 35 U.S.C. § 286 to any infringement not committed more than six years prior to the filing of the Counterclaim for infringement.

SIXTH AFFIRMATIVE DEFENSE

(No Willful Patent Infringement)

At least because Thorne has not infringed any valid claim of the '506 Patent, Thorne has not willfully infringed any claim of the '506 Patent.

SEVENTH AFFIRMATIVE DEFENSE

(Not an Exceptional Case)

If ChromaDex is entitled to any remedy, it is not entitled to a finding that this case is exceptional under 35 U.S.C. § 285, the Court's inherent authority, or any other basis, and therefore not entitled to an award of attorneys' fees or costs.

EIGHTH AFFIRMATIVE DEFENSE**(Failure to Mark)**

To the extent ChromaDex has failed to properly mark any of their relevant products or have failed to require others to mark any relevant products, as required by 35 U.S.C. § 287, no damages shall be recovered by ChromaDex in this action for infringement.

NINTH AFFIRMATIVE DEFENSE**(Equitable Doctrines)**

The Counterclaim is barred in whole or in part by the doctrines of equitable estoppel, patent misuse, implied license, patent exhaustion, disclaimer, and/or waiver.

COUNTER-COUNTERCLAIMS

Without admitting any of the allegations of Defendant and Counterclaim-Plaintiff ChromaDex, Inc.'s ("ChromaDex") other than those expressly admitted herein, and without prejudice to the right to plead additional counter-counterclaims, Plaintiffs, Counterclaim Defendants, and Counter-counterclaim Plaintiffs Thorne Research, Inc. ("Thorne Research") and Thorne HealthTech, Inc. ("Thorne HealthTech") (collectively, "Thorne") hereby counter-counterclaims against Defendants ChromaDex, Inc. ("ChromaDex"), Niagen Bioscience, Inc. ("Niagen"), and The Queen's University of Belfast ("QUB") (collectively, "Defendants") and allege as follows:

1. This is an action for declaratory judgment of invalidity and unenforceability of U.S. Patent No. 12,252,506 (the "'506 Patent") arising under the Declaratory Judgment Act (28 U.S.C. §§ 2201–2202) and the patent laws of the United States (35 U.S.C. § 1, et seq.).

2. This action arises from Defendants' campaign to unlawfully restrain Thorne's ability to sell its products. Defendants sent letters demanding that Thorne remove its products from the marketplace, made allegations of patent infringement, and filed a complaint through Amazon's

Patent Evaluation Express (“APEX”) procedure seeking removal of Thorne’s product listings. Defendants’ allegations are without merit. Thorne brings this action to obtain a judicial declaration that the ’506 Patent claims are invalid and unenforceable.

THE PARTIES

3. Thorne Research is a South Carolina corporation with its principal place of business at 620 Omni Industrial Blvd., Summerville, South Carolina 29486.

4. Thorne HealthTech is a Delaware corporation with its principal place of business at 620 Omni Industrial Blvd., Summerville, South Carolina 29486. Thorne Research is a wholly owned subsidiary of Thorne HealthTech.

5. On information and belief, ChromaDex is a California corporation with its principal place of business at 10900 Wilshire Boulevard, Suite 600, Los Angeles, California 90024. On information and belief, ChromaDex is a wholly owned subsidiary of Niagen.

6. On information and belief, Niagen is a Delaware corporation with its principal place of business at 10900 Wilshire Boulevard, Suite 600, Los Angeles, California 90024.

7. On information and belief, the Queen’s University of Belfast is a public research university in Belfast, Northern Ireland, United Kingdom, whose address is University Road, Belfast BT7 INN, United Kingdom.

JURISDICTION AND VENUE

8. Thorne incorporates by reference the preceding paragraphs as if fully set forth herein.

9. Thorne incorporates by reference the allegations set forth in Paragraphs 42 through 48 of the Complaint (Dkt. No. 1) as if fully set forth herein, to the extent those allegations remain consistent with the current record.

10. This Court has subject matter jurisdiction over Thorne's claims pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202, as well as the patent laws of the United States, 35 U.S.C. § 1, *et seq.*

11. Venue is proper for Defendants because Defendants have not raised an objection to venue.

12. This Court has personal jurisdiction over Defendants because Defendants did not object to personal jurisdiction.

13. Venue is proper because this counterclaim arises from the same transaction or occurrence as the claims in this action, and Defendants have submitted to the jurisdiction and venue of this Court, including ChromaDex by asserting its counterclaims herein.

14. Joinder of the Defendants is proper pursuant to Rules 19 and/or 20 of the Federal Rules of Civil Procedure and 35 U.S.C. § 299 because the conduct alleged herein raises an immediate, real, substantial, and justiciable controversy between Thorne and Defendants as to whether the '506 Patent is invalid, arises out of the same transaction, occurrence, or series of transactions or occurrences, and poses questions of fact common to all Defendants.

COUNTER-COUNTERCLAIM COUNT I

Declaratory Judgment of Invalidity of the '506 Patent

15. The foregoing allegations of this counter-counterclaim are incorporated by reference as if fully set forth herein.

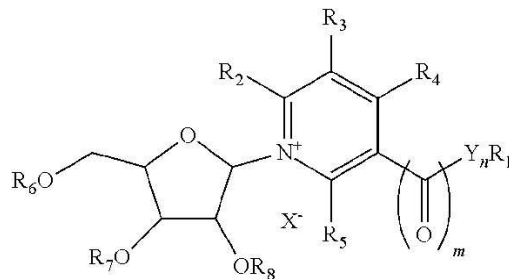
16. The claims of the '506 Patent are invalid because they fail to meet one or more conditions of patentability set forth in Title 35 of the U.S. Code, including, but not limited to, 35 U.S.C. §§ 101, 102, 103, and/or 112.

17. Nicotinamide riboside is an oral supplement that is a precursor for nicotinamide

adenine dinucleotide (NAD⁺), a vitamin B3 derivative produced naturally by the body.

18. The title of the '506 Patent is “Methods of Preparing Nicotinamide Riboside and Derivatives Thereof[.]”

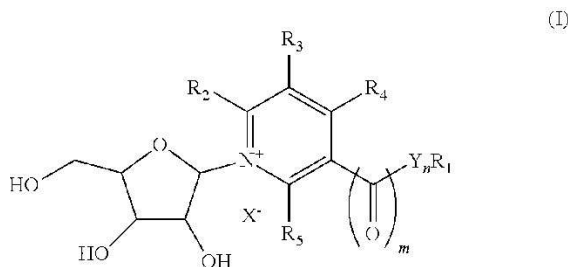
19. Independent claim 1 of the '506 Patent recites “[a] compound of formula (IV)” having the following structure:



20. Independent claim 1 of the '506 Patent recites a compound of formula (IV) where “R₆, R₇ and R₈, which may be the same or different, are each independently H or a hydroxyl-protecting group[.]”

21. Asserted claims 2, 3, 10-12, and 26 depend from claim 1 and thus, recite each and every element of claim 1.

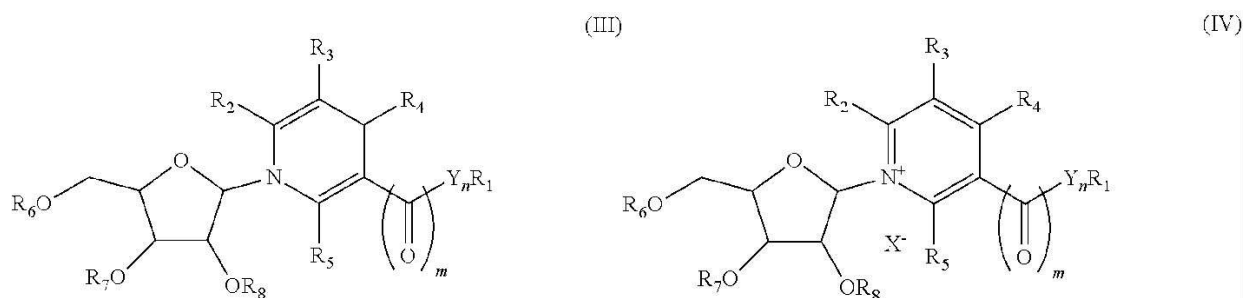
22. The specification of the '506 Patent describes a method of preparing nicotinamide riboside (“NR⁺”) salts of formula (I):



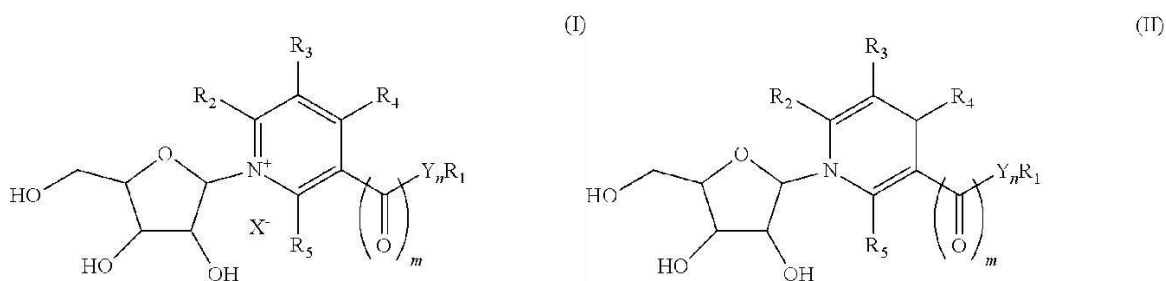
23. The specification of the '506 Patent states that the compound of formula (IV) is used as a precursor molecule to prepare the compound of formula (III) to produce the compound of formula (I):

- (a) “FIG. 2 depicts Scheme A, which is a scheme illustrating . . . that compounds of formula (IV) may be used to prepare compounds of formula (III), . . . ; that compounds of formula (III) may be used to prepare compounds of formula (II), . . . ; and that compounds of formula (II) may be used to prepare compounds of formula (I) . . . ” (*id.* at 12:13-20);
- (b) “[T]he compound of formula (III) is prepared by reacting a compound of formula (IV)” (*id.* at 8:14-15); and
- (c) “The methods for the preparation of compounds of formula (I) starting from compounds of formula (IV) are also very efficient and produce very good yields[.]” (*id.* at 10:63-65).

24. The specification of the '506 Patent states that the compounds of formula (III) and formula (IV) have R-groups R_6 , R_7 and R_8 :



25. The specification of the '506 Patent states that the compounds of formula (I) and formula (II) have the following structures:



26. The specification of the '506 Patent states that the compounds of formula (I) and

formula (II) do not contain R-groups R₆, R₇ or R₈.

27. The specification of the '506 Patent does not state that the compound of formula (I) is an example of the compound of formula (IV).

28. The specification of the '506 Patent does not state that the compound of formula (II) is an example of the compound of formula (IV).

29. The specification of the '506 Patent describes the compound of formula (IV) as having “hydroxyl protecting groups R₆, R₇ and R₈[.]” *Id.* at 8:66-67.

30. Hydrogen is not a hydroxyl protecting group.

31. The '506 Patent does not teach or disclose that hydrogen is a hydroxyl protecting group.

32. If R₆ is a hydrogen in the compound of formula (IV), then R₆ is not a hydroxyl protecting group.

33. If R₇ is a hydrogen in the compound of formula (IV), then R₇ is not a hydroxyl protecting group.

34. If R₈ is a hydrogen in the compound of formula (IV), then R₈ is not a hydroxyl protecting group.

35. The specification of the '506 Patent states that the “hydroxyl protecting groups R₆, R₇ and R₈, are required to be lipophilic” in the compound of formula (IV). *Id.* at 8:66-67.

36. The specification of the '506 Patent states “[t]he protecting groups used in the preparation of compounds of formula (III) from compounds of formula (IV) may advantageously be chosen to be sufficiently lipophilic so that they facilitate the migration of the compounds of formula (III) into the organic phase of the reaction medium for ease of extraction.” *Id.* at 11:14-19.

37. The specification of the '506 Patent describes the compound of formula (IV) where “at least two of R₆, R₇ and R₈ comprise unsubstituted acetyl[.]” *Id.* at 8:40-44.

38. The specification of the '506 Patent exemplifies the compound of formula (IV) where “n is 0, m is 1, R₁ is NH₂, R₂-R₅ are each H, R₆-R₈ are each acetyl, and X⁻ is ⁻OTf.” *Id.* at 9:8-10.

39. The specification of the '506 Patent describes the compound of formula (IV) “in the form of the triflate salt[.]” (*id.* at 10:42-44) and exemplifies: (A) “triacyetyl nicotinamide riboside, triflate (CF₃SO₃⁻, also known as —OTf) salt” (*id.* at 12:64-66); (B) “triacyetyl O-ethyl nicotinate riboside, triflate (—OTf) salt” (*id.* at 13:66-67); and (C) “tribenzoyl nicotinamide riboside, triflate (—OTf) salt” (*id.* at 14:58-60).

40. The specification of the '506 Patent does not contain any working examples of the compound of formula (IV) where R₆, R₇ or R₈ is a hydrogen.

41. The specification of the '506 Patent does not describe the compound of formula (IV) where R₆ is not a hydroxyl protecting group.

42. The specification of the '506 Patent does not describe the compound of formula (IV) where R₇ is not a hydroxyl protecting group.

43. The specification of the '506 Patent does not describe the compound of formula (IV) where R₈ is not a hydroxyl protecting group.

44. The specification of the '506 Patent does not describe the compound of formula (IV) where R₆ is a hydrogen.

45. The specification of the '506 Patent does not describe the compound of formula (IV) where R₇ is a hydrogen.

46. The specification of the '506 Patent does not describe the compound of

formula (IV) where R_8 is a hydrogen.

47. Claim 1's recitation that " R_6 , R_7 and R_8 , which may be the same or different, are each independently H" in the compound of formula (IV) is not described in the specification of the '506 Patent.

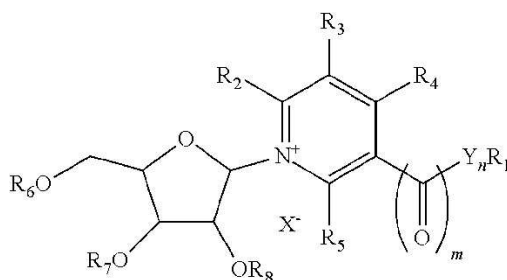
48. Claim 1 of the '506 Patent lacks written description and is invalid under 35 U.S.C. § 112.

49. Claim 1 of the '506 Patent lacks enablement and is invalid under 35 U.S.C. § 112.

50. Claims 2-3, 10-12, and 26 depend from claim 1 and thus, recite each and every element of claim 1. Therefore, claims 2-3, 10-12, and 26 of the '506 Patent also lack written description and enablement, and are invalid under 35 U.S.C. § 112.

51. The '506 Patent was filed on April 26, 2023 as U.S. Application No. 18/307,733 ("733 Application").

52. Claim 1 filed on April 26, 2023 in the '733 Application recites "[a] compound of formula (IV):



... where: ... R_6 , R_7 and R_8 , which may be the same or different, are each independently H."

53. The compound of formula (IV) having R_6 , R_7 and R_8 as hydrogen appeared for the first time, at the earliest, with the April 26, 2023 filing date of the '733 Application that issued as the '506 Patent.

54. The '506 Patent claims are entitled to priority no earlier than April 26, 2023.

55. U.S. Patent Application Publication No. 2016/0168184 (“’184 Publication”) published on June 16, 2016. Ex. 1.

56. The ’184 Publication is prior art to the asserted claims in the ’506 Patent.

57. The ’184 Publication anticipates the asserted claims in the ’506 Patent for the reasons described in IPR2026-00400; thus, the asserted claims in the ’506 Patent are invalid under 35 U.S.C. § 102.

58. The asserted claims in the ’506 Patent are obvious variations that are conventional in light of the prior art available for the reasons described in IPR2026-00400; thus, the asserted claims in the ’506 Patent are invalid under 35 U.S.C. § 103.

59. Thorne is entitled to a judicial declaration that the claims of the ’506 Patent are invalid.

PRAYER FOR RELIEF

WHEREFORE, Thorne respectfully requests that the Court enter judgment in its favor against Counter-Defendants as follows:

- A. A declaration, pursuant to 28 U.S.C. §§ 2201 and 2202, that one or more claims of U.S. Patent No. 12,252,506 (the “’506 Patent”), including but not limited to claims 1–3, 10–12, and 26, are invalid for failure to comply with one or more requirements of Title 35 of the United States Code, including without limitation 35 U.S.C. §§ 101, 102, 103, and 112;
- B. A declaration that the asserted claims of the ’506 Patent are invalid for lack of written description and/or lack of enablement under 35 U.S.C. § 112(a);
- C. A declaration that the asserted claims of the ’506 Patent are invalid as indefinite under 35 U.S.C. § 112(b);

- D. A declaration that the asserted claims of the '506 Patent are invalid as anticipated and/or obvious under 35 U.S.C. §§ 102 and/or 103 in view of the prior art;
- E. A declaration that this is an exceptional case under 35 U.S.C. § 285, and an award to Thorne of its reasonable attorneys' fees incurred in connection with this action;
- F. An award to Thorne of its costs of suit incurred herein, pursuant to 28 U.S.C. § 1920 and Fed. R. Civ. P. 54(d);
- G. An award of pre-judgment and post-judgment interest to the extent permitted by law; and
- H. Such other and further relief as the Court deems just and proper.

Respectfully submitted,

/s/Addie K. S. Ries

Addie K. S. Ries

S.C. Bar No. 103016

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Dated: September 2, 2026

CERTIFICATE OF SERVICE

I hereby certify that on September 2, 2026, a copy of the foregoing document was electronically filed and served upon all attorneys of record via the Court's CM/ECF system.

/s/Addie K. S. Ries

EXHIBIT 1

US 20160168184A1

(19) **United States**(12) **Patent Application Publication**
MIGAUD et al.(10) **Pub. No.: US 2016/0168184 A1**(43) **Pub. Date: Jun. 16, 2016**(54) **METHODS OF PREPARING NICOTINAMIDE RIBOSIDE AND DERIVATIVES THEREOF**(71) Applicant: **THE QUEEN'S UNIVERSITY OF BELFAST**, Belfast Antrim (GB)(72) Inventors: **Marie MIGAUD**, Lurgan Armagh (GB); **Philip REDPATH**, Portadown, Armagh (GB); **Kerri CROSSEY**, Magherafelt, County Derry (GB); **Mark DOHERTY**, Newtownabbey, County Antrim (GB)(21) Appl. No.: **14/908,831**(22) PCT Filed: **Jul. 24, 2014**(86) PCT No.: **PCT/EP2014/065971**

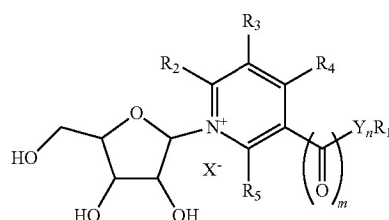
§ 371 (c)(1),

(2) Date: **Jan. 29, 2016**(30) **Foreign Application Priority Data**

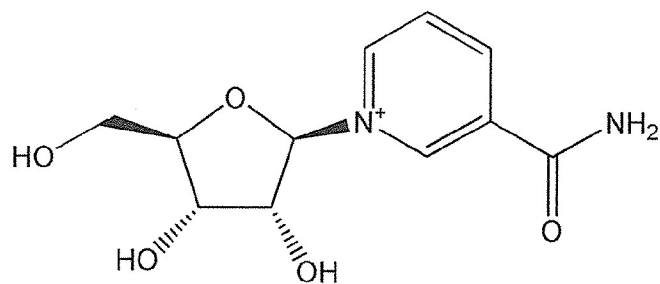
Jul. 29, 2013 (GB) 1313465.5

Publication Classification(51) **Int. Cl.****C07H 19/048** (2006.01)**C07H 1/06** (2006.01)(52) **U.S. Cl.**CPC **C07H 19/048** (2013.01); **C07H 1/06** (2013.01)(57) **ABSTRACT**

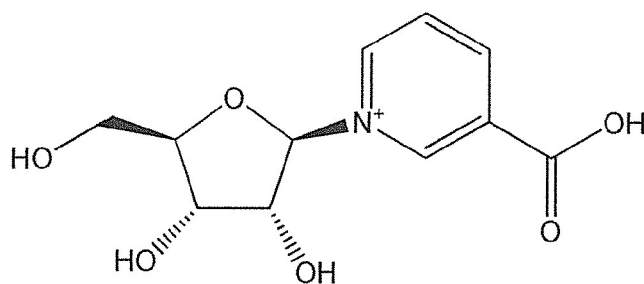
The invention relates to methods of preparing nicotinamide riboside and derivatives thereof. In an aspect, the invention relates to a method of preparing a compound of formula (I), wherein n is 0 or 1; m is 0 or 1; Y is O or S; R₁ is selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted primary or secondary amino, and substituted or unsubstituted azido; R₂-R₅, which may be the same or different, are each independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or substituted or unsubstituted alkynyl, and substituted or unsubstituted aryl; and X⁻ is an anion, selected from an anion of a substituted or unsubstituted carboxylic acid, a halide, a substituted or unsubstituted sulfonate, a substituted or unsubstituted phosphate, a substituted or unsubstituted sulfate, a substituted or unsubstituted carbonate, and a substituted or unsubstituted carbamate.



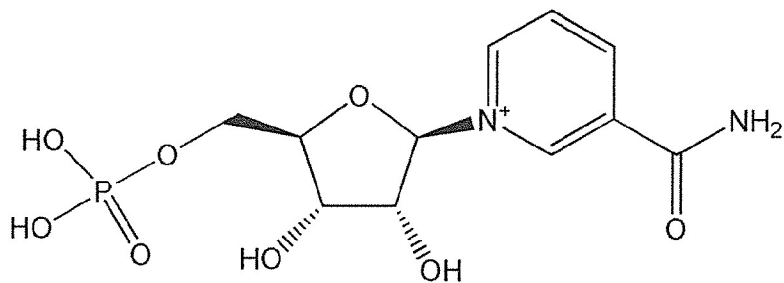
(I)



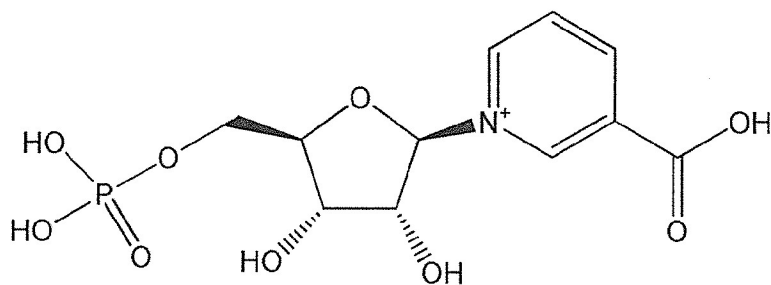
Nicotinamide riboside



Nicotinate riboside

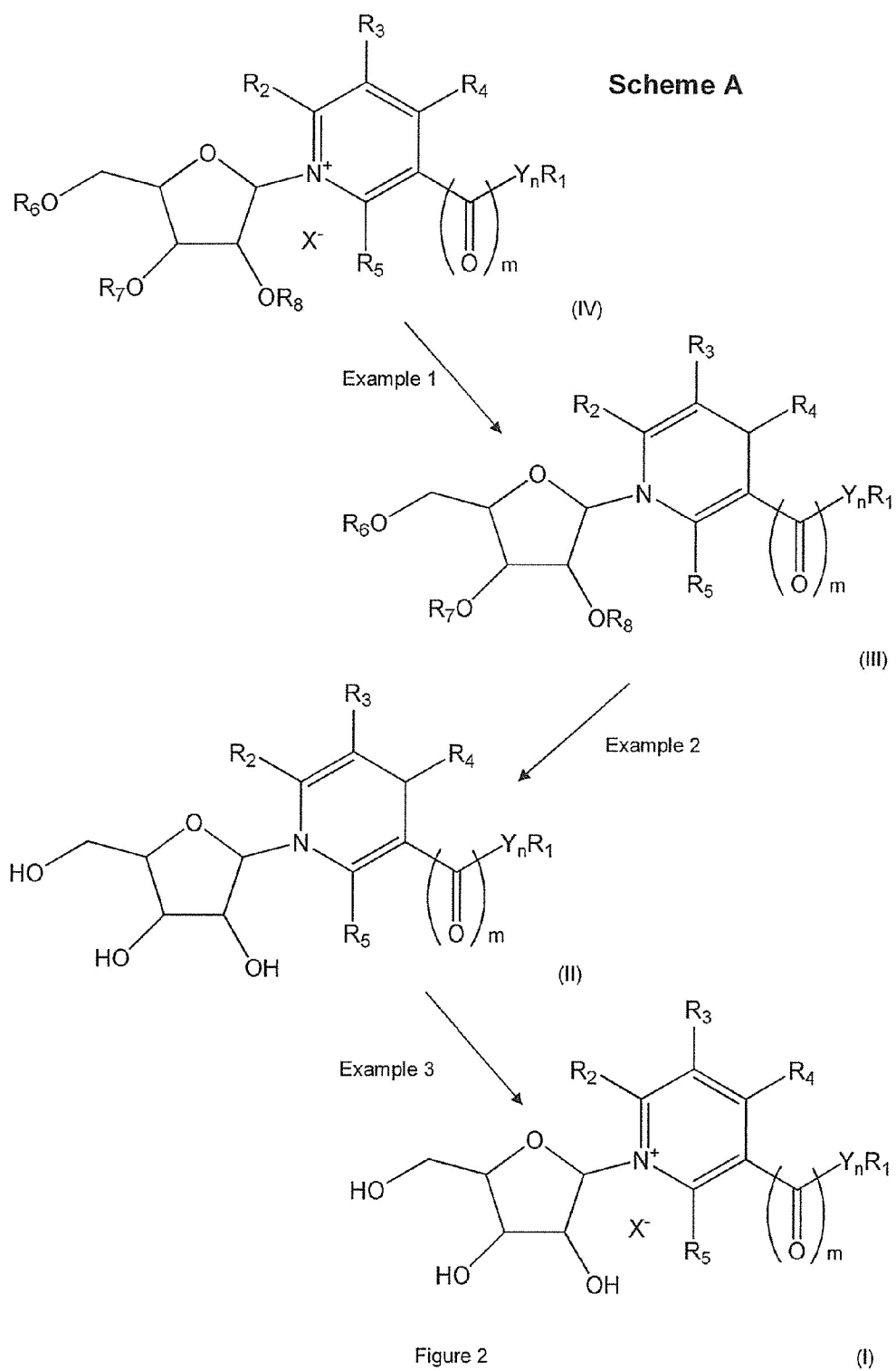


Nicotinamide mononucleotide



Nicotinate mononucleotide

Figure 1



Scheme B

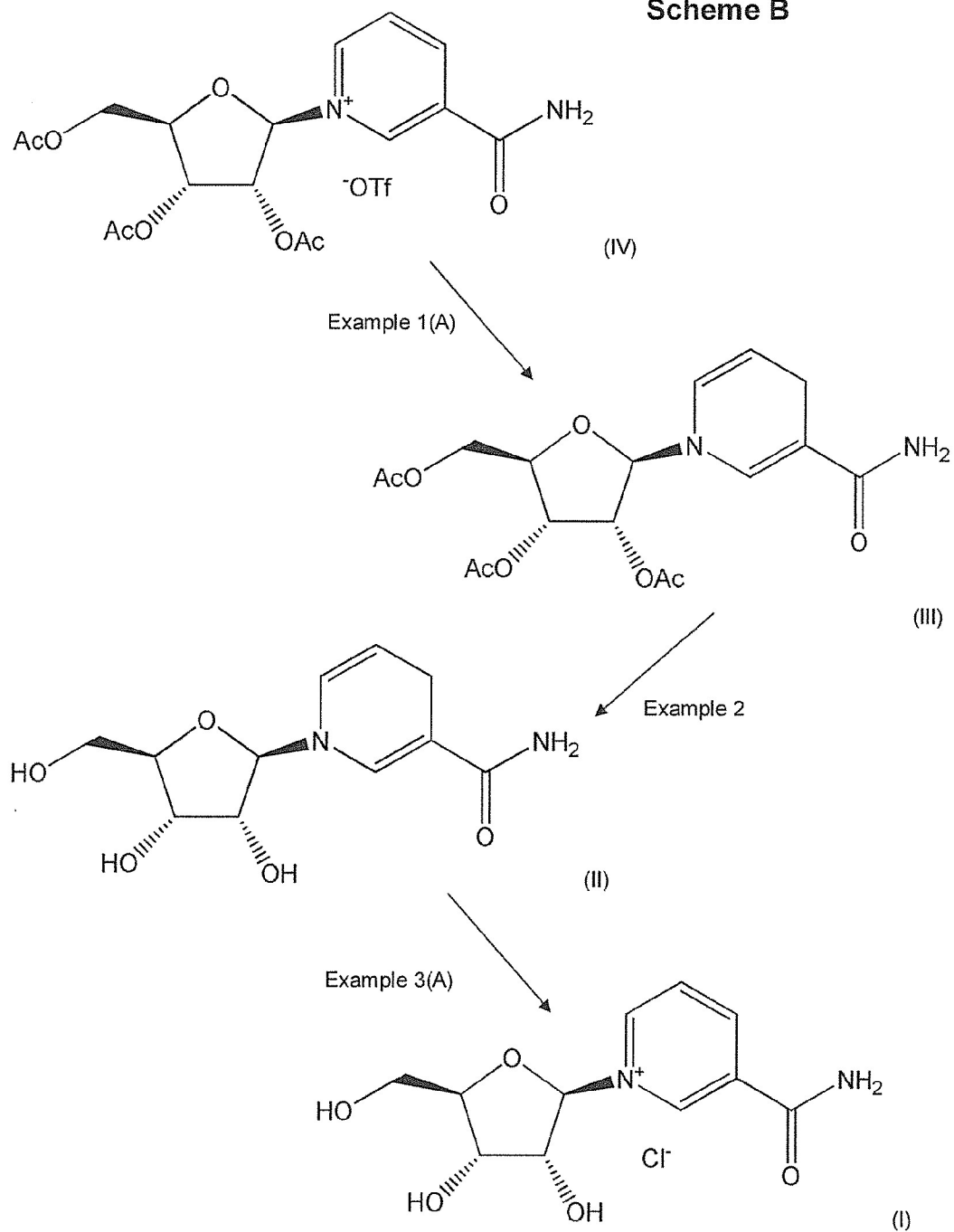
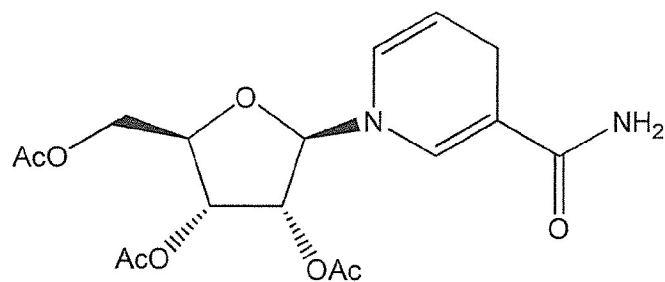
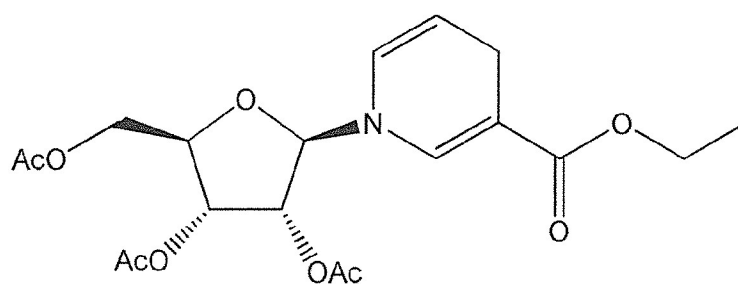


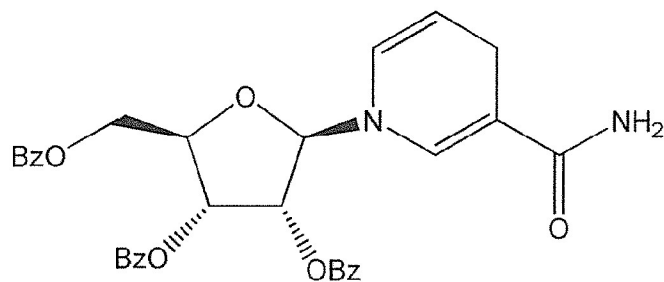
Figure 3



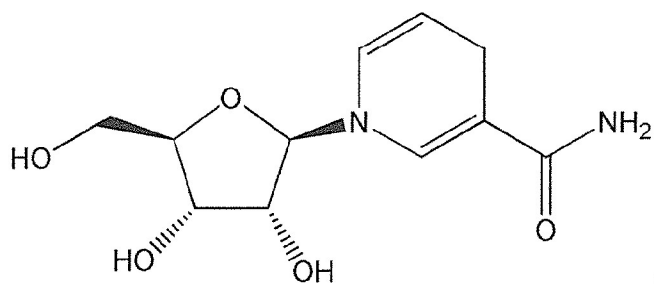
triacetyl-1,4-dihydronicotinamide riboside



triacetyl O-ethyl-1,4-dihydronicotinate riboside

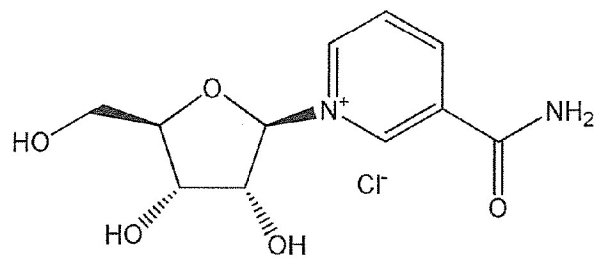


tribenzoyl-1,4-dihydronicotinamide riboside

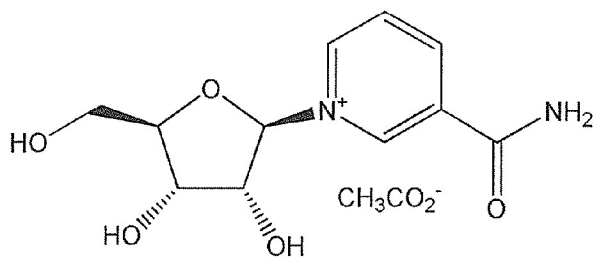


reduced nicotinamide riboside
(1-(beta-D-ribofuranosyl)-1,4-dihydronicotinamide)

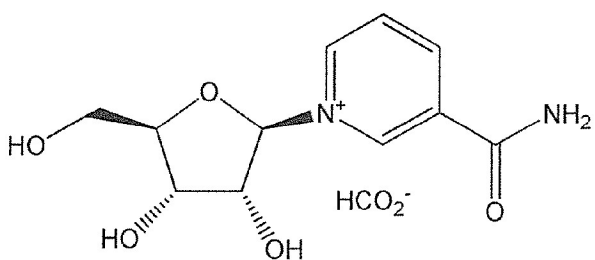
Figure 4



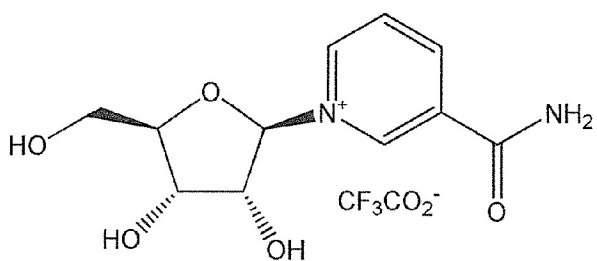
nicotinamide riboside, chloride salt



nicotinamide riboside, acetate salt



nicotinamide riboside, formate salt



nicotinamide riboside, trifluoroacetate salt

Figure 5

US 2016/0168184 A1

Jun. 16, 2016

1

METHODS OF PREPARING NICOTINAMIDE RIBOSIDE AND DERIVATIVES THEREOF

FIELD OF THE INVENTION

[0001] The invention relates to methods of preparing nicotinamide riboside and derivatives thereof.

BACKGROUND TO THE INVENTION

[0002] Nicotinamide riboside and derivatives thereof, including nicotinate riboside, nicotinamide mononucleotide and nicotinate mononucleotide, are metabolites of nicotinamide adenine dinucleotide (NAD⁺). The β -anomer forms of nicotinamide riboside, nicotinate riboside, nicotinamide mononucleotide and nicotinate mononucleotide are shown, without counter ions, in FIG. 1. As a NAD⁺ precursor, nicotinamide riboside has been shown in mice to enhance oxidative metabolism and protect against high-fat diet induced obesity, which has resulted in significant interest in nicotinamide riboside and its derivatives. Since nicotinamide riboside is a naturally occurring compound, nicotinamide riboside and its derivatives have great potential as natural, nutritional supplements, which may provide health benefits without causing side effects. One limitation in the commercial exploitation of nicotinamide riboside and derivatives thereof, as nutritional supplements, or otherwise, is that known synthetic protocols for preparing nicotinamide riboside and derivatives thereof have disadvantages, rendering them unsuitable for scaling up for commercial or industrial use.

[0003] WO 2007/061798 describes a method for the preparation of nicotinamide riboside and derivatives thereof. However, the disclosed method has a number of disadvantages. For example, trimethylsilyl trifluoromethanesulfonate (TMSOTf) is used as the catalyst in the disclosed method, and results in the prepared compounds inevitably being in the form of their triflate (OTf) salts. The triflate salt form of nicotinamide riboside, or derivatives thereof, is not suitable for use as a nutritional supplement, because of its associated toxicity. Thus, the compounds produced by the disclosed method are not suitable for use as they are prepared, and require an additional step to exchange the triflate anion for an anion that would be pharmaceutically acceptable and therefore suitable for commercialisation, utilizing for example, reverse phase liquid chromatography as disclosed. Additionally, nicotinamide riboside is chemically labile, in particular under the chromatographic conditions used in the disclosed method. It is therefore proposed that the chromatographic conditions used could result in batches of less than optimum purity and, within the batches, great variability in terms of the side products produced. Another disadvantage is that careful control of the temperature of the reaction is necessary to minimise decomposition in the final stages of the synthesis of nicotinamide riboside, yet the disclosed method is exothermic and is therefore prone to microenvironment thermal fluctuation, especially in the event of large scale production set up.

[0004] Tanimori et al (S. Tanimori, T. Ohta and M. Kirihaata, *Bioorganic & Medicinal Chemistry Letters*, 2002, 12, 1135-1137) and Franchetti et al (P. Franchetti, M. Pasqualini, R. Petrelli, M. Ricciutelli, P. Vita and L. Cappellacci, *Bioorganic & Medicinal Chemistry Letters*, 2004, 14, 4655-4658) also describe methods for the preparation of nicotinamide riboside. However, these methods also have the disadvantage of

inevitably resulting in the preparation of the triflate salt by virtue of using TMSOTf as catalyst.

[0005] In summary, the disclosed methods have disadvantages which present obstacles to the scaling up of the method for commercial or industrial use, and which, therefore, greatly limit the commercial opportunities for the methods and the resultant compounds.

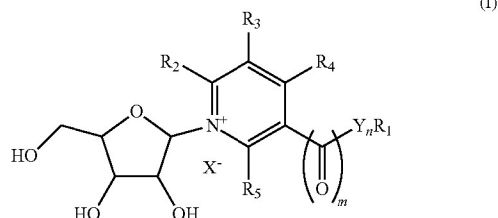
[0006] It is therefore an object of the invention to avoid or mitigate the disadvantages of the prior art.

[0007] It is also an object of the invention to provide a novel, useful and efficient method for the preparation of nicotinamide riboside and derivatives thereof.

[0008] It is also an object of the invention to provide a method for the preparation of nicotinamide riboside and derivatives thereof, whereby the method may be used to introduce a counter ion of choice to the prepared compounds, thereby producing compounds suitable for use as nutritional supplements or otherwise.

SUMMARY OF THE INVENTION

[0009] According to the present invention, there is provided a method of preparing a compound of formula (I)



[0010] wherein

[0011] n is 0 or 1;

[0012] m is 0 or 1;

[0013] Y is O or S;

[0014] R₁ is selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted primary or secondary amino, and substituted or unsubstituted azido;

[0015] R₂-R₅ which may be the same or different, are each independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, and substituted or unsubstituted aryl;

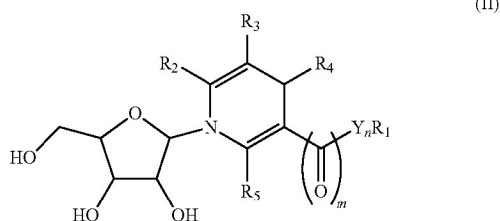
[0016] X⁻ is an anion, selected from an anion of a substituted or unsubstituted carboxylic acid, a halide, a substituted or unsubstituted sulfonate, a substituted or unsubstituted phosphate, a substituted or unsubstituted sulfate, a substituted or unsubstituted carbonate, and a substituted or unsubstituted carbamate;

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[0017] comprising reacting a compound of formula (II)



[0018] wherein n, m, Y and R₁-R₅ are as defined above;

[0019] with a compound of the formula Z⁺X⁻,

[0020] wherein X⁻ is as defined above, and

[0021] wherein Z⁺ is a N-containing cation;

[0022] in the presence of an aqueous solution and a carbon-containing catalyst;

[0023] to form the compound of formula (I).

[0024] Optionally, Z⁺ is selected from a substituted or unsubstituted ammonium, a substituted or unsubstituted pyridinium, a substituted or unsubstituted pyrrolidinium, a substituted or unsubstituted imidazolium and a substituted or unsubstituted triazolium.

[0025] Optionally, Z⁺ is a substituted or unsubstituted ammonium of the formula N⁺HR^IR^{II}R^{III}, wherein R^I, R^{II} and R^{III}, which may be the same or different, are each independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, and substituted or unsubstituted aryl.

[0026] Optionally, Z⁺ is an unsubstituted ammonium of the formula NH₄⁺.

[0027] Optionally, X⁻ is an anion of a substituted or unsubstituted carboxylic acid selected from an anion of a substituted or unsubstituted monocarboxylic acid and an anion of a substituted or unsubstituted dicarboxylic acid.

[0028] Optionally, X⁻ is an anion of a substituted monocarboxylic acid, further optionally an anion of a substituted propanoic acid or an anion of a substituted acetic acid. Optionally, X⁻ is an anion of substituted propanoic acid, further optionally an anion of a hydroxy propanoic acid, still further optionally an anion of 2-hydroxypropanoic acid, being lactic acid, the anion of lactic acid being lactate. Optionally, X⁻ is an anion of a substituted acetic acid, being a substituted acetate, further optionally a trihaloacetate selected from trichloroacetate, tribromoacetate and trifluoroacetate. Still further optionally, the trihaloacetate is trifluoroacetate.

[0029] Optionally, X⁻ is an anion of an unsubstituted monocarboxylic acid selected from formic acid, acetic acid, propionic acid and butyric acid, being formate, acetate, propionate and butyrate, respectively.

[0030] Optionally, X⁻ is an anion of a substituted or unsubstituted amino-monocarboxylic acid or an anion of a substituted or unsubstituted amino-dicarboxylic acid. Further optionally, X⁻ is an anion of an amino-dicarboxylic acid, optionally selected from glutamic acid and aspartic acid, being glutamate and aspartate, respectively.

[0031] Optionally, X⁻ is an anion of ascorbic acid, being ascorbate.

[0032] Optionally, X⁻ is a halide selected from chloride, bromide, fluoride and iodide, further optionally chloride or bromide.

[0033] Optionally, X⁻ is a substituted or unsubstituted sulfonate. Further optionally, X⁻ is a trihalomethanesulfonate selected from trifluoromethanesulfonate, tribromomethanesulfonate and trichloromethanesulfonate. Still further optionally, the trihalomethanesulfonate is trifluoromethanesulfonate.

[0034] Optionally, X⁻ is a substituted or unsubstituted carbonate, further optionally hydrogen carbonate.

[0035] Optionally, X⁻ is selected from chloride, acetate, formate, trifluoroacetate, ascorbate, aspartate, glutamate and lactate. Further optionally, X⁻ is selected from chloride, acetate, formate and trifluoroacetate.

[0036] Optionally, the compound of the formula Z⁺X⁻ is selected from ammonium chloride, ammonium acetate, ammonium formate, ammonium trifluoroacetate, ammonium ascorbate, ammonium aspartate, ammonium glutamate and ammonium lactate. Further optionally, the compound of the formula Z⁺X⁻ is selected from ammonium chloride, ammonium acetate, ammonium formate and ammonium trifluoroacetate.

[0037] Optionally, the compound of formula (II) and the carbon-containing catalyst are present in a respective molar ratio of from about 10:1 to about 1:10, optionally from about 5:1 to about 1:5, further optionally from about 4:1 to about 1:4, still further optionally about 1:1 or 1:2 or 1:3 or 1:4.

[0038] Suitable carbon-containing catalysts include, but are not limited to, activated charcoal or graphite.

[0039] As used herein, the term "activated charcoal" is intended to mean a carbon containing material processed to be highly porous thereby increasing the surface area of the material. The term "activated charcoal" is intended to be synonymous with the term "activated carbon". The activated charcoal may be in the form of powders and/or fibres and/or granules and/or pellets. Optionally, the activated charcoal may act as a support for a metal. Suitable metals include, but are not limited to, transition metals. Suitable transition metals include, but are not limited to the platinum group metals, optionally selected from ruthenium, rhodium, palladium, osmium, iridium, and platinum, or a combination thereof.

[0040] Optionally, the aqueous solution consists essentially of water.

[0041] Optionally, the aqueous solution comprises, in addition to water, an organic solvent.

[0042] Suitable organic solvents include, but are not limited to, substituted or unsubstituted ethers, substituted or unsubstituted esters, substituted or unsubstituted ketones, substituted or unsubstituted aliphatic or aromatic hydrocarbons, and combinations thereof.

[0043] Optionally, the organic solvent, when present, comprises an ether selected from diethyl ether, methyl tert-butyl ether, ethyl tert-butyl ether, di-tert-butyl ether, diisopropyl ether, dimethoxymethane, tetrahydrofuran, 2-methyltetrahydrofuran, and tetrahydropyran, or a combination thereof.

[0044] Optionally, the organic solvent, when present, comprises an ester selected from methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate and n-butyl acetate, or a combination thereof.

[0045] Optionally, the organic solvent, when present, comprises a ketone selected from methyl isobutyl ketone and methyl isopropyl ketone, or a combination thereof.

[0046] Optionally, the organic solvent, when present, comprises an unsubstituted aliphatic hydrocarbon solvent selected from pentane, hexane, cyclohexane and heptane, or a combination thereof.

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[0047] Optionally, the organic solvent, when present, comprises a substituted aliphatic hydrocarbon solvent, optionally a halogenated aliphatic hydrocarbon solvent, further optionally a chlorinated aliphatic hydrocarbon solvent selected from dichloromethane, trichloromethane, tetrachloromethane, 1,2-chloroethane, 1,1,1-trichloroethane and trichloroethylene, or a combination thereof.

[0048] Optionally the organic solvent, when present, comprises an aromatic hydrocarbon solvent selected from benzene, toluene, ethylbenzene and xylene, or a combination thereof.

[0049] Optionally, the aqueous solution comprises water and organic solvent in a respective ratio by volume of from about 1:5 to about 5:1, optionally from about 1:3 to about 3:1, further optionally from about 1:2 to about 2:1, still further optionally about 1:1.

[0050] Optionally, the reaction is carried out in a pH range of from about 6 to about 8, optionally from about 6.5 to about 7.5.

[0051] Optionally, the reaction is carried out at a temperature of from about 10° C. to about 40° C., optionally from about 15° C. to about 35° C., further optionally from about 15° C. to about 30° C., still further optionally from about 15° C. to about 20° C., even further optionally from about 20° C. to about 25° C., even further optionally at a temperature of about 20° C. or 21° C. or 22° C. or 23° C. or 24° C. or 25° C.

[0052] Optionally, the reaction is carried out for a period of time of from about 1 minute to about 180 minutes, optionally, from about 2 minutes to about 120 minutes, further optionally from about 5 minutes to about 120 minutes, still further optionally from about 10 minutes to about 120 minutes, even further optionally from about 20 minutes to about 120 minutes, even further optionally from about 30 minutes to about 120 minutes, still further optionally from about 60 minutes to about 120 minutes, even further optionally from about 60 minutes to about 90 minutes, still further optionally about 60 minutes or 70 minutes or 80 minutes.

[0053] Optionally, the method further comprises a filtration step to remove the carbon-containing catalyst from the prepared compound of formula (I). Suitable filtration means for use in the filtration step include, but are not limited to, syringe filters and/or paper filters, and/or any inert, insoluble substance capable of acting as a filter, e.g. alumina and/or silica and/or diatomaceous earth. It will be appreciated any other suitable filtration means may be used.

[0054] As used herein, the term “substituted” is intended to mean that any one or more hydrogen atoms is replaced with any suitable substituent, provided that the normal valency is not exceeded and the replacement results in a stable compound. Suitable substituents include, but are not limited to, alkyl, alkylaryl, aryl, heteroaryl, halide, hydroxyl, carboxylate, carbonyl (including alkylcarbonyl and arylcarbonyl), phosphate, amino (including alkylamino, dialkylamino, hydroxylamino, dihydroxylamino, alkyl hydroxylamino, arylamino, diarylamino and alkylaryl amino), thiol (including alkylthiol, arylthiol and thiocarboxylate), sulfate, nitro, cyano and azido.

[0055] As used herein, the term “alkyl” is intended to mean a substituted or unsubstituted, saturated or unsaturated, optionally saturated, linear, branched or cyclic, aliphatic hydrocarbon, having from 1 to 12 carbon atoms, optionally from 1 to 10 carbon atoms, further optionally from 1 to 8 carbon atoms, still further optionally from 1 to 6 carbon atoms, even still further optionally 1 or 2 or 3 or 4 or 5 carbon

atoms. Suitable alkyls include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-pentyl, iso-pentyl, n-hexyl, iso-hexyl, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Optionally, when Y is O, n is 1, and m is 1, ethyl is preferred.

[0056] As used herein, the term “alkenyl” is intended to mean a substituted or unsubstituted, linear, branched or cyclic, aliphatic hydrocarbon, having at least one carbon-carbon double bond, and having from 2 to 12 carbon atoms, optionally from 2 to 10 carbon atoms, further optionally from 2 to 8 carbon atoms, still further optionally from 2 to 6 carbon atoms, even still further optionally 2 or 3 or 4 or 5 carbon atoms. Suitable alkenyl groups include, but are not limited to, ethenyl, propenyl and butenyl.

[0057] As used herein, the term “alkynyl” is intended to mean a substituted or unsubstituted, linear, branched or cyclic, aliphatic hydrocarbon, having at least one carbon-carbon triple bond, and having from 2 to 12 carbon atoms, optionally from 2 to 10 carbon atoms, further optionally from 2 to 8 carbon atoms, still further optionally from 2 to 6 carbon atoms, even still further optionally 2 or 3 or 4 or 5 carbon atoms. Suitable alkynyl groups include, but are not limited to, ethynyl, propynyl, butynyl, and the like.

[0058] As used herein, the term “aryl” is intended to mean a substituted, unsubstituted, monocyclic or polycyclic, aromatic hydrocarbon. Suitable aryls include, but are not limited to, substituted or unsubstituted phenyl, and substituted or unsubstituted heteroaryl.

[0059] Optionally, the substituted or unsubstituted primary or secondary amino is selected from substituted or unsubstituted alkylamino, substituted or unsubstituted dialkylamino, substituted or unsubstituted hydroxylamino, substituted or unsubstituted dihydroxylamino, and substituted or unsubstituted alkyl hydroxylamino.

[0060] Optionally, the substituted or unsubstituted azido is selected from substituted or unsubstituted alkyl azido and substituted or unsubstituted aryl azido.

[0061] It will be appreciated that, when n is 0 and m is 0, R₁ is directly attached to the pyridine ring or to the pyridinium ring, as appropriate.

[0062] Optionally, in an embodiment of formula (I), n is 0, m is 1, R₁ is NH₂, R₂-R₅ are each H, and X⁻ is selected from chloride, acetate, formate and trifluoroacetate.

[0063] Optionally, in an embodiment of formula (II), n is 0, m is 1, R₁ is NH₂, and R₂-R₅ are each H.

[0064] Optionally, the compound of formula (II) and the compound of the formula Z⁺X⁻ are present in a respective molar ratio of from about 1:5 to about 5:1, optionally from about 1:3 to about 3:1, further optionally from about 1:2 to about 2:1, still further optionally about 1:1.

[0065] Optionally, the method comprises stirring the reactants, optionally using a magnetic or mechanical stirrer, further optionally an overhead mechanical stirrer.

[0066] In an embodiment, the carbon-containing catalyst used in the preparation of a compound of formula (I) may be provided in the form of an activated charcoal column, for example an activated charcoal material such as those supplied by Sigma Aldrich under the trade names NORIT (Trade Mark) or DARCO (Trade Mark), or from CarboChem, W Lancaster Ave, Ardmore, Pa. 19003, USA, or a carbon supported catalyst in a CatCart Packer (Trade Mark) column from ThalesNano, Graphisoft Park, Záhony u. 7. H-1031 Budapest, Hungary. In this embodiment, the activated charcoal column may be used as part of any suitable liquid chro-

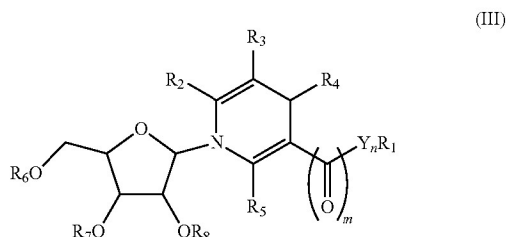
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matography system, including, but not limited to, a fast protein liquid chromatography (FPLC) or a high performance liquid chromatography (HPLC) system, or a flow chemistry system, such as the ThalesNano (Trade Mark) H-cube systems and related flow reactors, available from ThalesNano, details provided above. In this case, the reactants would be recirculated onto the column in a continuous manner until the compound of formula (II) is no longer detected by UV at 340 nm.

[0067] Optionally, the compound of formula (II) is prepared by reacting a compound of formula (III)



[0068] wherein

[0069] n, m, Y and R₁-R₅ are as defined above; and

[0070] R₆, R₇ and R₈, which may be the same or different, are each independently a hydroxyl-protecting group;

[0071] with a deprotecting agent;

[0072] to form the compound of formula (II).

[0073] Suitable R₆, R₇ and R₈ moieties include, but are not limited to, ester-type protecting groups, ether-type protecting groups, and silyl-type protecting groups.

[0074] As used herein, the term "ester-type protecting group" is intended to mean a protecting group that forms an ester bond for the purpose of hydroxyl protection and which may be substituted or unsubstituted. Suitable ester-type protecting groups include, but are not limited to, acetyl, propionyl, isopropionyl, benzoyl, and trihaloacetyl, optionally trifluoroacetyl or trichloroacetyl.

[0075] As used herein, the term "ether-type protecting group" is intended to mean a protecting group that forms an ether bond for the purpose of hydroxyl protection and which may be substituted or unsubstituted. Suitable ether-type protecting groups include, but are not limited to, benzyl, p-methoxybenzyl, methoxymethyl and allyl ethers.

[0076] As used herein, the term "silyl-type protecting group" refers to a protecting group that forms a silyloxy bond for the purpose of hydroxyl protection. Examples thereof from trimethylsilyl, triethylsilyl, triisopropylsilyl, 2-(trimethylsilyl)ethoxymethyl, tert-butyldimethylsilyl, tert-butyl-diphenylsilyl and tetraisopropylsilyl.

[0077] Optionally, the R₆, R₇ and R₈ moieties are selected from substituted and unsubstituted acetyl, and substituted and unsubstituted benzoyl.

[0078] Optionally, at least two of R₆, R₇ and R₈ are selected from unsubstituted acetyl or unsubstituted benzoyl.

[0079] Optionally the deprotecting agent is an acid or a base. Deprotection can also be achieved by catalytic hydrogenation (Pd/C; H₂) for the aromatic ether protecting groups and by fluoride-catalysed chemistry (e.f. TBAF in THF) for all the silyl ethers. Optionally, when R₆, R₇ and R₈ each comprise unsubstituted acetyl or unsubstituted benzoyl, the deprotecting agent is a base, optionally selected from NH₃,

Na₂CO₃ and NaOH. It will be appreciated by a skilled person that any other conventional deprotecting agent may be used.

[0080] Optionally, the reaction is carried out in the presence of a protic or aprotic solvent or a combination thereof.

[0081] Suitable protic solvents include, but are not limited to, water, substituted or unsubstituted alcohol, or a combination thereof. Suitable substituted alcohols include substituted or unsubstituted fluorinated alcohols. Suitable unsubstituted alcohols include methanol, ethanol and propanol, optionally methanol.

[0082] Suitable aprotic organic solvents include, but are not limited to, substituted or unsubstituted ethers, substituted or unsubstituted esters, substituted or unsubstituted ketones, substituted or unsubstituted aliphatic or aromatic hydrocarbons, and combinations thereof, as defined above.

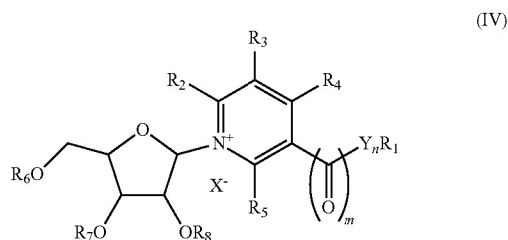
[0083] Optionally, the reactants are subjected to mechanical grinding, further optionally using a ball milling or planetary ball milling machine.

[0084] Optionally, in an embodiment of formula (III), n is 0, m is 1, R₁ is NH₂, R₂-R₅ are each H, and R₆-R₈ are each acetyl.

[0085] Further optionally, in another embodiment of formula (III), n is 1, Y is 0, m is 1, R₁ is ethyl, R₂-R₅ are each H, and R₆-R₈ are each acetyl.

[0086] Still further optionally, in another embodiment of formula (III), n is 0, m is 1, R₁ is NH₂, R₂-R₅ are each H, and R₆-R₈ are each benzoyl.

[0087] Optionally, the compound of formula (III) is prepared by reacting a compound of formula (IV)



[0088] wherein

[0089] n, m, Y, R₁-R₈ and X⁻ are as defined above;

[0090] with a reducing agent,

[0091] an aqueous solution

[0092] and an organic solvent

[0093] to form a compound of formula (III).

[0094] Optionally, X— is selected from ascorbate, glutamate, aspartate, lactate and acetate.

[0095] Suitable organic solvents are as defined above in respect of the preparation of a compound of formula (I) from formula (II).

[0096] Optionally, when at least two of R₆, R₇ and R₈ comprise unsubstituted acetyl, the organic solvent is selected from dichloromethane, 1,2-chloroethane, n-butyl acetate, chloroform and ethyl acetate, or a combination thereof, further optionally ethyl acetate.

[0097] Optionally, when at least two of R₂, R₃ and R₄ comprise unsubstituted benzoyl, the organic solvent is selected from trichloroethylene, carbon tetrachloride, diisopropyl ether, toluene, methyl tert-butyl ether, benzene and diethyl ether, or a combination thereof, further optionally diethyl ether.

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[0098] Optionally, the reducing agent is selected from sodium dithionite or sodium borohydride.

[0099] Optionally, the method may comprise the simultaneous addition of the reducing agent, aqueous solution and organic solvent; or the sequential addition of the reducing agent, aqueous solution and organic solvent, in any order; or a combination thereof.

[0100] Optionally, the aqueous solution consists essentially of water.

[0101] It will be appreciated that, optionally, the aqueous solution and the organic solvent form a bi-phasic solution comprising an aqueous phase and an organic phase.

[0102] Optionally, the method comprises the additional steps of

[0103] separating the organic phase from the aqueous phase; and

[0104] extracting the compound of formula (III) from the organic solvent.

[0105] It will be appreciated by a skilled person that the hydroxyl protecting groups R_6 , R_7 and R_8 , are required to be lipophilic to the extent that the reduced compound of formula (III), once prepared, migrates into the organic phase of the bi-phasic reaction medium formed by the aqueous solution (aqueous phase) and organic solvent (organic phase).

[0106] Optionally, the reactants are subjected to mechanical grinding, further optionally using a ball mill or planetary ball milling machine.

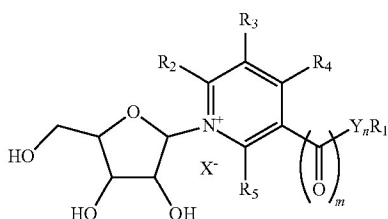
[0107] Optionally, in an embodiment of formula (IV), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, R_6 - R_8 are each acetyl, and X^- is ^-OTf .

[0108] Further optionally, in another embodiment of formula (III), n is 1, Y is O, m is 1, R_1 is ethyl, R_2 - R_5 are each H, R_6 - R_8 are each acetyl, and k is ^-OTf .

[0109] Still further optionally, in another embodiment of formula (III), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, and R_6 - R_8 are each benzoyl, and X^- is ^-OTf .

[0110] According to the invention, there are also provided compounds derivable from the methods disclosed herein.

[0111] Accordingly to the invention, there is further provided a compound of formula (I)



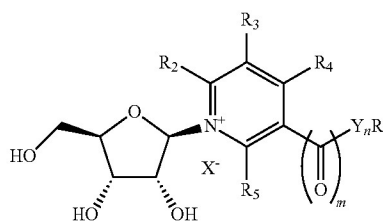
(I)

[0112] wherein

[0113] n , m , Y , R_1 - R_5 and X^- are as defined above.

[0114] Optionally, X^- is selected from acetate, formate and trifluoroacetate.

[0115] Optionally, the compound of formula (I) has the formula (IA), i.e. is the β -anomer,

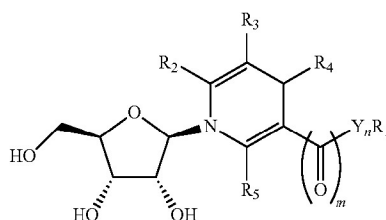


(IA)

[0116] wherein

[0117] n , m , Y , R_1 - R_5 and X^- are as defined above.

[0118] Optionally, the compound of formula (II) has the formula (IIA), i.e. is the β -anomer,

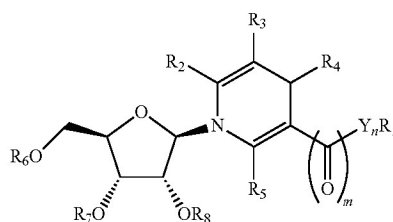


(IIA)

[0119] wherein

[0120] n , m , Y and R_1 - R_5 are as defined above.

[0121] Optionally, the compound of formula (III) has the formula (IIIA), i.e. is the β -anomer,

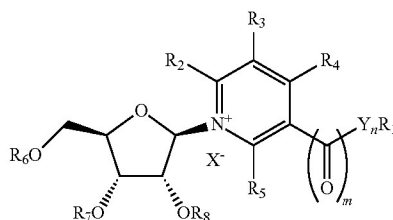


(IIIA)

[0122] wherein

[0123] n , m , Y and R_1 - R_8 are as defined above.

[0124] Optionally, the compound of formula (IV) has the formula (IVA), i.e. is the β -anomer,



(IVA)

[0125] wherein

[0126] n , m , Y , R_1 - R_8 and X^- are as defined above.

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[0127] Advantages of the invention include, but are not limited to the following:

[0128] (1) The preparation of compounds of formula (I) from compounds of formula (II) provides an efficient method of introducing a counter ion of choice for nicotinamide riboside and its derivatives. Starting from a compound of formula (II), e.g. reduced NRH, a desired counter ion may be introduced. Furthermore, even if the method starts by using compounds of formula (IV) in the form of the triflate salt, the methods of the invention enable the triflate anion to be exchanged during the method in a simple and efficient manner, to a counter ion of choice. Thus, the disclosed methods conveniently enable the preparation of compounds having potential use as nutritional supplements or otherwise.

[0129] (2) The invention provides stereoselective methods for the preparation of nicotinamide riboside and derivatives thereof, producing the desired β -anomer. This is in contrast, for example, to Tanimori et al, which is not stereoselective and produces significant amounts of the α -anomer, which is undesirable. Additionally, the methods of the invention are useful, efficient, and can be easily scaled up for industry and commercialisation, and provide for the minimisation of solvent use, purification and reaction time. For example, the methods of the invention for preparing compounds of formula (I) from compounds of formula (II), are conveniently completed in less than 2 hours with quantitative yields. The methods for the preparation of compounds of formula (I) starting from compounds of formula (IV) are also very efficient and produce very good yields. The methods may conveniently be carried out at room temperature.

[0130] (3) The methods described herein are capable of preparing not just nicotinamide riboside but also a whole range of derivatives, which is not disclosed in either Tanimori et al or Franchetti et al. The derivatives include not just derivatives of nicotinamide riboside but also the reduced form of nicotinamide riboside and derivatives thereof. Furthermore, although not described herein, a skilled person will appreciate that, starting from compounds of formula (II), it is possible to easily access the phosphorylated parents of nicotinamide riboside and derivatives thereof, for example nicotinamide mononucleotide and nicotinate mononucleotide.

[0131] (4) The protecting groups used in the preparation of compounds of formula (III) from compounds of formula (IV) may advantageously be chosen to be sufficiently lipophilic so that they facilitate the migration of the compounds of formula (III) into the organic phase of the reaction medium, for ease of extraction.

[0132] (5) The methods described herein conveniently use reactants which enable the compounds of formula (I) to be prepared in a neutral pH range of from about 6 to about 8. For example, in the preparation of the compounds of formula (I) from the compounds of formula (II), this neutral pH range enables both the starting materials (compounds of formula (II)), which are acid labile, and the final products (compounds of formula (I)), which are base labile, to be stable during the reaction.

[0133] (6) The inventors have surprisingly found that, the use of a N-containing cation as Z^+ (the proton source), conveniently enables the efficient preparation of compounds of formula (I) from compounds of formula (II) in quantitative yield, and, as mentioned in point

(5), in a neutral pH range. Without wishing to be bound by theory, it is proposed that Z^+ , the proton source, must be a conjugated acid of an organic base which is protonated in an equilibrated manner within the neutral pH range. The inventors propose that, by using a N-containing cation as the proton source Z^+ , the N atom of the proton source has a pKa greater than the pKa of the N atom of the dihydropyridine of the compound of formula (II). Therefore, in simple terms, due to the relative pKa values, the N atom of the proton source Z^+ (i.e. $N-H^+$) holds onto the proton H^+ until after the N atom of the dihydropyridine has been oxidised (which oxidation, the inventors propose, is facilitated by the carbon-containing catalyst). It is only after the N atom of the dihydropyridine has been oxidised that it will be protonated by the proton source Z^+ . The inventors propose that if a proton source other than one containing a N atom is used, for example a phosphonium cation containing $P-H^+$ or a sulfonium cation containing $S-H^+$, it is proposed that such proton sources would cause the pH of the reaction medium to fall below the neutral pH range, and the proton sources would release their protons in this lower pH range. It is proposed that this change would cause the N atom of the dihydropyridine to be protonated before oxidation, thereby resulting in the undesirable hydrolysis of a C—N bond of the dihydropyridine. It is also proposed that this resultant instability of the dihydropyridine due to the undesirable breaking of a C—N bond, would also occur using a weak acid (e.g. carboxylic acid) or a strong acid (e.g. hydrochloric acid, phosphoric acid or sulphuric acid). Thus, it is proposed that only a N-containing cation as proposed, is capable of releasing a proton in a pH range (neutral) which allows the reaction to proceed as desired to form the compounds of formula (I) from the compounds of formula (II).

EXAMPLES

[0134] Embodiments of the present invention will now be described, with reference to the accompanying, non-limiting examples and drawings, in which:

[0135] FIG. 1 shows the β -anomer forms of nicotinamide riboside, nicotinate riboside, nicotinamide mononucleotide and nicotinate mononucleotide, without counter ions;

[0136] FIG. 2 depicts Scheme A, which is a scheme illustrating, in general terms, that compounds of formula (IV) may be used to prepare compounds of formula (III), as described in Example 1; that compounds of formula (III) may be used to prepare compounds of formula (II), as described in Example 2; and that compounds of formula (II) may be used to prepare compounds of formula (I), as described in Example 3; wherein n, m, Y, R_1 - R_8 and X^- are as defined above;

[0137] FIG. 3 depicts Scheme B, which is a scheme illustrating, in general terms, that triacetyl nicotinamide riboside, triflate salt may be used to prepare triacetyl-1,4-dihydronicotinamide riboside, as described in Example 1(A), and that triacetyl-1,4-dihydronicotinamide riboside may be used to prepare 1-(β -D-ribofuranosyl)-1,4-dihydronicotinamide, as described in Example 2, and that 1-(β -D-ribofuranosyl)-1,4-dihydronicotinamide may be used to prepare nicotinamide riboside, chloride salt, as described in Examples 3(A), 3(E) and 3(F). The β -anomers of all of the mentioned

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compounds are shown. It will be appreciated that Scheme B is merely exemplary and is not to be construed as limiting the invention thereto;

[0138] FIG. 4 shows the β -anomer forms of triacetyl-1,4-dihydronicotinamide riboside (Example 1(A)), triacetyl O-ethyl-1, 4-dihydronicotinamide riboside, (Example 1(B)), tribenzoyl-1,4-dihydronicotinamide riboside (Example 1(C)), and 1-(β -D-ribofuranosyl)-1,4-dihydronicotinamide (Example 2); and

[0139] FIG. 5 shows the β -anomer forms of nicotinamide riboside, chloride salt (Examples 3(A), 3(E) and 3(F)), nicotinamide riboside, acetate salt (Example 3(B)), nicotinamide riboside, formate salt (Example 3(C)), and nicotinamide riboside, trifluoroacetate salt (Example 3(D)).

EXAMPLE 1

[0140] Compounds of formula (III) were prepared in accordance with the invention as follows. The pH of the reaction media described in the following examples was in the region of about 6-8.

Example 1(A)

Preparation of Reduced Triacetyl Nicotinamide Riboside, Namely Triacetyl-1,4-dihydronicotinamide riboside, a Compound of Formula (III) (the β -anomer Form of which is Shown in FIG. 4)

[0141] Reduction: All solvents were degassed prior to use by sonication and argon bubbling. Sodium dithionite (0.656 g, 3.76 mmol, 2 eq) and sodium hydrogencarbonate (0.79 g, 9.40 mmol, 5 eq) were added to a clean, dry round bottom flask with a magnetic stirrer and placed under inert atmosphere. A compound of formula (IV), namely triacetyl nicotinamide riboside, triflate (CF_3SO_3^- , also known as —OTf) salt (1 g, 1.88 mmol, 1 eq) was then dissolved in a minimum amount of water (<10 ml) and slowly added to the reaction vessel. Once the reaction settled, further water was added to the reaction until all of the reactants had dissolved (<10 ml) and was left to stir for 20 minutes. The aqueous solution was then extracted with three half portions of dichloromethane (DCM). The DCM fractions were collected and concentrated under reduced pressure, affording the triacetyl-1,4-dihydronicotinamide riboside derivative (triacetyl-NRH) with residual amounts of starting material (<5%). The aqueous layer was subjected to the above conditions a second time to increase yields which averaged 65%. Ethyl acetate was also an excellent alternative extraction solvent in place of DCM, yielding a 75% yield.

[0142] $^1\text{H-NMR}$ (MeOD, 400 MHz)- δ 7.15 (s, 1H, H-5), 5.95 (d, 1H, J=7.21 Hz, H-6), 5.25 (d, 1H, J=2.84 Hz) & 5.17 (d, 1H, J=1.80 Hz) (H-8 & H-7), 4.96 (d, 1H, J=7.09 Hz, H-4), 4.87 (ABX, 1H, $J_{a,a'}=8.18$ Hz, $J_{a,b}=3.60$ Hz, H-9), 4.26 (d, 2H, J=3.20 Hz, H-10 & H-10'), 4.19 (m, 1H, J=3.00 Hz, H-3), 3.13 (m, 2H, J=1.18 Hz, H-2), 2.13 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H) (H-13, H-15, H-17). $^{13}\text{C-NMR}$ (MeOD, 125 MHz)- δ 172.80 (C-11), 170.40 (C-12, C-14, C-16), 137.90 (C-5), 125.20 (C-4), 105.12 (C-6), 95.24 (C-3), 83.49 (C-9), 71.18 (C-8), 70.26 (C-7), 61.55 (C-10), 22.16 (C-2), 21.52 (C-13, C-15, C-17). HMRS m/z: 383.1445; Calc. Mass: 383.1454.

[0143] The compound of formula (IV), namely triacetyl nicotinamide riboside, triflate (—OTf) salt was prepared as follows. Nicotinamide (10 g, 81.89 mmol, 1 eq) was silylated using TMSCI (15.6 ml, 122.85 mmol, 1.5 eq) in HMDS (100

ml) at 130° C. in quantitative yield, in order to force the β -selectivity via the following Vorbrüggen reaction. Ribose tetraacetate (also known as tetraacetate riboside) was reacted with the resultant silylated nicotinamide in the presence of 5 equivalents of TMSOTf. The reactants were shaken in a 1.5 ml steel vessel with a 5 mm diameter steel ball bearing in a Retsch MM400 mixer mill at 25 Hz for 0.5 h. At this point the formed triacetylated nicotinamide riboside (compound of formula (IV)) could be isolated. It will be appreciated that the triacetyl nicotinamide riboside is not limited to being produced by this exact method, and could, for example, be produced using a conventional Vorbrüggen reaction as described, for example, in International PCT patent publication no. WO 2007/061798 or in T. Yang, N. Y. K. Chan and A. A. Sauve, *Journal of Medicinal Chemistry*, 2007, 50, 6458-6461.

[0144] $^1\text{H-NMR}$ (MeOD, 400 MHz)- δ 9.61 (s, 1H, aromatic), 9.30 (dt, 1H, J=6.3, 1.4 Hz, aromatic), 9.10 (dt, 1H, J=8.2, 1.4 Hz, aromatic), 8.37 (dd, 1H, J=8.2, 6.3 Hz, aromatic), 6.60 (d, 1H, J=3.9 Hz, H-1 (anomeric)), 5.60 (dd, 1H, J=5.6, 3.9 Hz, H-2), 5.46 (t, 1H, J=5.6 Hz, H-3), 4.81-4.84 (m, 1H, H-4), 4.61 (ABX, 1H, $J_{a,a'}=13.1$ Hz, $J_{a,b}=3.5$ Hz, H-5), 4.51 (ABX, 1H, $J_{a,a'}=13.0$ Hz, $J_{a,b}=2.8$ Hz, H-5'), 2.20 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.16 (s, 3H, OAc).

[0145] $^{13}\text{C-NMR}$ (MeOD, 125 MHz)- δ 172.1, 171.6, 171.2 ($3\times\text{C}=\text{OCH}_3$), 164.9 ($\text{C}=\text{ONH}_2$), 147.0, 144.3, 142.3, 136.2, 129.6, (aromatic), 121.6 (q, J=320.2 Hz, CF_3), 99.4 (C-1 (anomeric)), 84.4 (C-4), 77.6 (C-2), 70.7 (C-3), 63.5 (C-5), 20.7 (OAc), 20.3 (OAc), 20.2 (OAc).

[0146] $^{19}\text{F-NMR}$ (MeOD, 376 MHz)- δ -79.9 (triflate counterion)

Example 1(B)

Preparation of Reduced Triacetyl Nicotinate Ester Riboside, Namely Triacetyl O-ethyl-1,4-dihydronicotinamide riboside, a Compound of Formula (III) (the 3-anomer Form of which is Shown in FIG. 4)

[0147] Reduction: A compound of formula (IV), namely triacetyl O-ethyl nicotinate riboside, triflate (—OTf) salt (2.30 g, 4.2 mmol, 1 eq) was dissolved in 20 mL H_2O and a solution of a solution of NaHCO_3 (1.77 g, 21.0 mmol, 5 eq) and sodium dithionite (1.47 g, 8.22 mmol, 2 eq) in 30 mL H_2O was added and stirred for 2 hrs. The yellow solution obtained was then washed with $2\times$ ethyl acetate (EtOAc, 40 mL), the organic layer dried over MgSO_4 , filtered and concentrated to provide 900 mg (39% yield) of 2,3,5-triacetyl O-ethyl-1,4-dihydronicotinamide riboside (a yellow oil) without further purification. 80% purity based on $^1\text{H-NMR}$.

[0148] $^1\text{H-NMR}$ - δ 7.27 (1H, s, H-6), 6.05 (1H, dd, J=8.2, 1.5 Hz, H-7), 5.26 (1H, dd, J=5.8, 2.8 Hz, H-3), 5.23 (1H, dd, J=6.9, 5.8 Hz, H-2), 5.08 (1H, d, J=6.9 Hz, H-1), 4.81 (1H, dt, J=8.3, 3.5 Hz, H-8), 4.24-4.30 (3H, m, H-4, H-5, H-5'), 4.11 (2H, q, J=7.2 Hz, H-11), 3.04-3.06 (2H, m, H-9), 2.16 (3H, s, OAc), 2.12 (3H, s, OAc), 2.09 (3H, s, OAc), 1.25 (3H, t, J=7.2 Hz, H-12).

[0149] $^{13}\text{C-NMR}$ - δ 172.2, 171.5, 171.3, 169.8, ($3\times\text{C}=\text{O}-\text{CH}_3$ and $\text{C}=\text{O}-\text{OEt}$), 139.9 (C-6), 126.3 (C-7), 106.4 (C-8), 101.5 (C-10), 94.2 (C-1), 80.4 (C-4), 72.3 (C-2), 72.1 (C-3), 64.8 (C-5), 61.0 (C-11), 23.4 (C-9), 20.7, 20.5, 20.3 ($3\times\text{C}=\text{O}-\text{CH}_3$), 14.8 (C-12).

[0150] The compound of formula (IV), namely triacetyl O-ethyl nicotinate riboside, triflate (—OTf) salt was prepared as follows. Ribose tetraacetate (also known as tetraac-

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etate riboside) was reacted with ethyl nicotinate (Sigma Aldrich) using the general ball milling Vorbrüggen procedure described in Example 1(A) above. The reactants, namely 1 eq tetraacetate riboside, 1 eq TMSOTf, 1 eq ethyl nicotinate, were reacted for 30 mins in a 1.5 ml steel vessel with a 1.5 cm diameter steel ball bearing in a Retsch MM400 mixer mill at 25 Hz. The crude reaction mixture (containing some unreacted ethyl nicotinate and starting sugar, <10%) was used for the reduction step (described above) without further purification. It will be appreciated that the triacetyl O-ethyl nicotinate riboside, triflate (—OTf) salt is not limited to being produced by this exact method, and could, for example, be produced using a conventional Vorbrüggen reaction as described, for example, in International PCT patent publication no. WO 2007/061798 or in T. Yang, N. Y. K. Chan and A. A. Sauve, *Journal of Medicinal Chemistry*, 2007, 50, 6458-6461.

[0151] ¹H-NMR (D₂O, 400 MHz)-δ 9.45 (s, 1H, aromatic), 9.14 (d, 1H, J=6.1 Hz, aromatic), 9.02 (d, 1H, J=7.8 Hz, aromatic), 8.18 (t, 1H, J=6.7 Hz, aromatic), 6.51 (d, 1H, J=4.1 Hz, H-1 (anomeric)), 5.47 (t, 1H, J=4.4 Hz, H-2), 5.36 (t, 1H, J=4.7 Hz, H-3), 4.81-4.84 (m, 1H, H-4), 4.45-4.48 (m, 2H, H-5), 4.36 (q, 2H, J=7.0 Hz, C=OCH₂CH₃), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.25 (t, 3H, J=7.0 Hz, C=OCH₂CH₃).

[0152] ¹³F-NMR (D₂O, 376 MHz)-δ -79.0 (triflate counterion)

Example 1(C)

Preparation of Reduced Tribenzoyl Nicotinamide Riboside, Namely

Tribenzoyl-1,4-dihydronicotinamide riboside, a Compound of Formula (III) (the β-anomer Form of which is Shown in FIG. 4)

[0153] Reduction (unoptimised): A compound of formula (IV), namely tribenzoyl nicotinamide riboside, triflate (—OTf) salt was dissolved in minimal methanol and transferred to a round bottomed flask, 10 mL of H₂O was added to the solution and most of the methanol removed via rotary evaporation. The starting material crashed out of solution and 20 mL of diethyl ether (Et₂O) was added until the solids solubilized into a biphasic system. A solution of NaHCO₃ (420 mg, 5 mmol, 5 eq) and sodium dithionite (348 mg, 2 mmol, 2 eq) in 10 mL H₂O was added and stirred for 2 hrs. The layers were separated and the ether layer was dried over MgSO₄ and concentrated to provide 428 mg (76% yield) of tribenzoyl-1,4-dihydronicotinamide riboside (yellow solid) without further purification. 80% purity based on ¹H-NMR. Pure material is obtained by Biotage purification.

[0154] ¹H-NMR-δ 8.01-8.04 (2H, m, OBz), 7.81-7.86 (4H, m, OBz), 7.25-7.55 (9H, m, OBz), 7.13 (1H, s, H-6), 6.01 (1H, dd, J=8.2, 1.5 Hz, H-7), 5.68 (1H, dd, J=6.2, 3.5 Hz, H-3), 5.57 (1H, dd, J=6.7, 6.2 Hz, H-2), 5.29 (1H, d, J=6.7 Hz, H-1), 4.61-4.68 (2H, m, H-8, H-5), 4.50-4.55 (2H, m, H-4, H-5'), 3.93-3.94 (2H, m, H-9). ¹³C-NMR-δ 172.7, 167.6, 166.7, 166.6 (3× C=O—C₆H₅, C=ONH₂), 138.1 (C-6), 134.9, 134.8, 134.6, 130.9, 130.8, 130.7, 130.3, 130.0, 129.8, 129.7 (3× OBz), 125.7 (C-7), 105.9 (C-8), 94.9 (C-1), 80.3 (C-4), 72.9 (C-2), 72.7 (C-3), 65.4 (C-5), 23.6 (C-9).

[0155] The compound of formula (IV), namely tribenzoyl nicotinamide riboside, triflate (—OTf) salt was prepared as follows. Ribose tetraacetate (also known as tetraacetate riboside) was reacted with TMS-nicotinamide (trimethylsilyl N-trimethylsilyl(pyridine-3-carboximide), available from

Sigma-Aldrich) using the general ball milling Vorbrüggen procedure described in Example 1(A) above. The reactants, namely 1 eq 1-acetate-tribenzoate riboside, 1 eq TMSOTf and 1 eq TMS-nicotinamide, were reacted for 30 mins in a 1.5 ml steel vessel with a 1.5 cm diameter steel ball bearing in a Retsch MM400 mixer mill at 25 Hz. 1 eq of DCE (dichloroethylene) was required and the crude reaction mixture (containing some unreacted nicotinamide and starting benzoate sugar, <10%) was used for the reduction step (described above) without further purification. It will be appreciated that the tribenzoyl nicotinamide riboside, triflate (—OTf) salt is not limited to being produced by this exact method, and could, for example, be produced using a conventional Vorbrüggen reaction as described, for example, in International PCT patent publication no. WO 2007/061798 or in T. Yang, N.Y. K. Chan and A. A. Sauve, *Journal of Medicinal Chemistry*, 2007, 50, 6458-6461.

[0156] ¹H-NMR (MeOD, 400 MHz)-δ 9.59 (s, 1H, aromatic), 9.31 (d, 1H, J=6.4 Hz, aromatic), 8.94 (d, 1H, J=8.1 Hz, aromatic), 8.15 (dd, 1H, J=8.1, 6.4 Hz, aromatic), 7.90-7.94 (m, 6H, OBz), 7.50-7.54 (m, 3H, OBz), 7.31-7.38 (m, 6H, OBz), 6.79 (d, 1H, J=3.9 Hz, H-1 (anomeric)), 5.97 (dd, 1H, J=5.6, 3.9 Hz, H-2), 5.87 (t, 1H, J=5.6 Hz, H-3), 5.13-5.16 (m, 1H, H-4), 4.83-4.91 (m, 2H, H-5).

[0157] ¹⁹F-NMR (MeOD, 376 MHz)-δ -79.1 (triflate counterion)

EXAMPLE 2

[0158] A compound of formula (II), namely NRH (reduced nicotinamide riboside, also known as 1-(β-D-ribofuranosyl)-1,4-dihydronicotinamide (the β-anomer form of which is shown in FIG. 4) was prepared as follows. The pH of the reaction medium described in the following example was in the region of about 6-8.

[0159] Reduced triacetyl nicotinamide riboside, namely triacetyl-1,4-dihydronicotinamide riboside, a compound of formula (III), prepared in Example 1(A) above, was deprotected using mechanochemical (MeOH, NaOH) processes to remove the acetyl moiety afforded NRH quantitatively. 100 mgs of (III) was dissolved in 0.5 mL of MeOH containing 0.05 g of NaOH. The compounds were reacted for 30 mins in a 1.5 ml steel vessel with a 1.5 cm diameter steel ball bearing in a Retsch MM400 mixer mill at 25 Hz.

[0160] ¹H-NMR (MeOD, 400 MHz)-δ 7.18 (s, 1H, H-5), 0.14(d, 1H, J=8.28 Hz, H-6), 4.85 (m, 1H, H-3), 4.76 (d, 1H, J=5.77 Hz, H-4), 4.04 (m, 2H, H-7&H-8), 3.93 (m, 1H, J=2.76, H-9), 3.72 (ABX, 1H, J_{aa}=12.55 Hz, J_{ab}=3.51 Hz, H-10), 3.65 (ABX, 1H, J_{aa}=12.55 Hz, J_{ab}=4.02 Hz, H-10'), 3.10 (q, 2H, J=1.51 Hz H-2). ¹³C-NMR (MeOD, 125 MHz)-δ 172.88 (C-11), 137.83 (C-5), 125.29 (C-4), 105.19 (C-6), 95.00 (C-3), 83.54 (C-9), 71.10 (C-8), 70.20 (C-7), 61.61 (C-10), 22.09 (C-2); HRMS m/z: 257.1130; Calc. Mass: 257.1137.

[0161] It will be appreciated that the deprotection step as described above may be used to deprotect any other compound of formula (III), including, but not limited to, reduced triacetyl nicotinate ester riboside, namely 2,3,5-triacetyl O-ethyl-1,4-dihydronicotininate riboside, prepared in Example 1(B), and reduced tribenzoyl nicotinamide riboside, namely tribenzoyl-1,4-dihydronicotinamide riboside, prepared in Example 1(C). The deprotection step may also be modified to suit particular requirements.

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EXAMPLE 3

[0162] Compounds of formula (I) were prepared in accordance with the invention as follows. The pH of the reaction media described in the following examples was in the region of about 6-8.

Example 3(A)

Preparation of Nicotinamide Riboside, Chloride Salt (the β -anomer Form of which is Shown in FIG. 5).

[0163] A compound of formula (II), namely NRH (reduced nicotinamide riboside, shown in FIG. 2; 50 mg, 0.20 mmol, 1 eq), was dissolved in 5 mL H₂O and then 1 eq (i.e. 0.20 mmol) of ammonium chloride was added in one portion. Activated charcoal (~10 mg, i.e. 0.80 mmol) was then added and the mixture stirred at RT for ~1 hr and then filtered and freeze-dried to give the chloride salt of nicotinamide riboside, quantitatively, i.e. 100% conversion and pure product.

[0164] ¹H-NMR (D₂O, 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (dt, 1H, J=6.3, 1.4 Hz, aromatic), 8.83 (dt, 1H, J=8.2, 1.4 Hz, aromatic), 8.13 (dd, 1H, J=8.2, 6.3 Hz, aromatic), 6.13 (d, 1H, J=4.3 Hz, H-1 (anomeric)), 4.37 (t, 1H, J=4.7 Hz, H-2), 4.31-4.34 (m, 1H, H-4), 4.21 (t, 1H, J=4.7 Hz, H-3), 3.90 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a,b}=3.5 Hz, H-5), 3.75 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a',b}=2.8 Hz, H-5').

[0165] It will be appreciated that the NRH may be that obtained in Example 2, or may be obtained commercially from e.g. Diverchim, 100, rue Louis Blanc, 60 765 Montataire Cedex, France—(CAS Registry Number: 19132-12-8) either as a pure product or as a mixture of anomers.

Example 3(B)

Preparation of Nicotinamide Riboside, Acetate Salt (the β -anomer Form of which is Shown in FIG. 5)

[0166] The method described in Example 3(A) was carried out, except that 1 eq (i.e. 0.20 mmol) of ammonium acetate was added. The acetate salt of nicotinamide riboside was obtained, quantitatively.

[0167] ¹H-NMR (D₂O, 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (d, 1H, J=6.3 Hz, aromatic), 8.83 (d, 1H, J=8.2 Hz, aromatic), 8.12 (m, 1H, aromatic), 6.09 (d, 1H, J=4.4 Hz, H-1 (anomeric)), 4.36 (t, 1H, J=4.7 Hz, H-2), 4.32-4.35 (m, 1H, H-4), 4.21 (t, 1H, J=4.7 Hz, H-3), 3.91 (ABX, 1H, J_{a,a'}=13.1 Hz, J_{a,b}=2.8 Hz, H-5), 3.75 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a',b}=3.5 Hz, H-5), 1.79 (s, 3H, OAc).

Example 3(C)

Preparation of Nicotinamide Riboside, Formate Salt (the β -anomer Form of which is Shown in FIG. 5)

[0168] The method described in Example 3(A) was carried out, except that 1 eq (i.e. 0.20 mmol) of ammonium formate (methanoate) was added. The formate salt of nicotinamide riboside was obtained, quantitatively.

[0169] ¹H-NMR (D₂O, 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (d, 1H, J=6.3 Hz, aromatic), 8.83 (d, 1H, J=8.2 Hz, aromatic), 8.29 (s, 1H, formate), 8.12 (m, 1H, aromatic), 6.09 (d, 1H, J=4.4 Hz, H-1 (anomeric)), 4.36 (t, 1H, J=4.7 Hz, H-2), 4.31-4.34 (m, 1H, H-4), 4.21 (t, 1H, J=4.7 Hz, H-3), 3.91 (ABX, J_{a,a'}=13.1 Hz, J_{a,b}=3.5 Hz, H-5), 3.79 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a',b}=2.8 Hz, H-5').

Example 3(D)

Preparation of Nicotinamide Riboside, trifluoroacetate Salt (the β -anomer Form of which is Shown in FIG. 5)

[0170] The method described in Example 3(A) was carried out, except that 1 eq (i.e. 0.20 mmol) of ammonium trifluoroacetate was added. The trifluoroacetate salt of nicotinamide riboside was obtained, quantitatively.

[0171] ¹H-NMR (D₂O, 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (d, 1H, J=6.3 Hz, aromatic), 8.83 (d, 1H, J=8.2, aromatic), 8.13 (dd, 1H, J=8.2, 6.3 Hz, aromatic), 6.13 (d, 1H, J=4.3 Hz, H-1 (anomeric)), 4.35 (t, 1H, J=4.7 Hz, H-2), 4.31-4.34 (m, 1H, H-4), 4.20 (t, 1H, J=4.7 Hz, H-3), 3.89 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a,b}=3.6 Hz, H-5), 3.74 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a',b}=2.9 Hz, H-5'). ¹⁹F-NMR (D₂O, 376 MHz)- δ -75.7 (CF₃COO⁻).

Example 3(E)

Preparation of Nicotinamide Riboside, Chloride Salt (the β -anomer form of which is Shown in FIG. 5)

[0172] An alternative method to that described in Example 3(A) was carried out as follows. NRH (reduced nicotinamide riboside, shown in FIG. 4; 50 mg, 0.20 mmol, 1 eq) was dissolved in 5 mL H₂O:EtOAc (1:1) and then 1 eq (i.e. 0.20 mmol) of ammonium chloride was added in one portion. Upon work-up after 1 hr, no oxidation had taken place and the starting materials were fully recovered. The recovered NRH and ammonium chloride were re-suspended in the same solvent system with addition of activated charcoal (~10 mg, i.e. 0.8 mmol) and stirred at RT for 1 hr. Subsequent filtration and freeze-drying afforded the chloride salt of nicotinamide riboside in quantitative yield. Thus it was concluded that a carbon-containing catalyst, e.g. activated charcoal, was essential to the method.

[0173] ¹H-NMR (D₂O, 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (dt, 1H, J=6.3, 1.4 Hz, aromatic), 8.83 (dt, 1H, J=8.2, 1.4 Hz, aromatic), 8.13 (dd, 1H, J=8.2, 6.3 Hz, aromatic), 6.13 (d, 1H, J=4.3 Hz, H-1 (anomeric)), 4.37 (t, 1H, J=4.7 Hz, H-2), 4.31-4.34 (m, 1H, H-4), 4.21 (t, 1H, J=4.7 Hz, H-3), 3.90 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a,b}=3.5 Hz, H-5), 3.75 (ABX, 1H, J_{a,a'}=13.0 Hz, J_{a',b}=2.8 Hz, H-5').

Example 3(F)

Preparation of Nicotinamide Riboside, Chloride Salt (the β -anomer Form of which is Shown in FIG. 5)

[0174] The method described in Example 3(E) was carried out, except that NRH (reduced nicotinamide riboside, shown in FIG. 4; 50 mg, 0.20 mmol, 1 eq) was dissolved in 5 mL H₂O:THF (1:1), instead of H₂O:EtOAc (1:1), and then 1 eq (i.e. 0.20 mmol) of ammonium chloride was added in one portion. Upon work-up after 1 hr, no oxidation had taken place and the starting materials were fully recovered. The recovered NRH and ammonium chloride were re-suspended in the same solvent system with addition of activated charcoal (~10 mg, i.e. 0.8 mmol) and stirred at RT for 1 hr. Subsequent filtration and freeze-drying afforded the chloride salt of nicotinamide riboside in quantitative yield. Thus it was concluded that a carbon-containing catalyst, e.g. activated charcoal, was essential to the method.

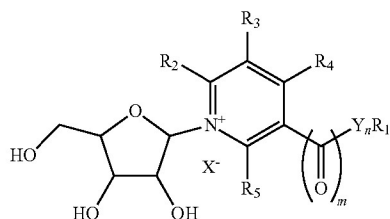
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[0175] $^1\text{H-NMR}$ (D_2O , 400 MHz)- δ 9.46 (s, 1H, aromatic), 9.12 (dt, 1H, $J=6.3, 1.4$ Hz, aromatic), 8.83 (dt, 1H, $J=8.2, 1.4$ Hz, aromatic), 8.13 (dd, 1H, $J=8.2, 6.3$ Hz, aromatic), 6.13 (d, 1H, $J=4.3$ Hz, H-1 (anomeric)), 4.37 (t, 1H, $J=4.7$ Hz, H-2), 4.31-4.34 (m, 1H, H-4), 4.21 (t, 1H, $J=4.7$ Hz, H-3), 3.90 (ABX, 1H, $J_{a,a'}=13.0$ Hz, $J_{a,b}=3.5$ Hz, H-5), 3.75 (ABX, 1H, $J_{a,a'}=13.0$ Hz, $J_{a',b}=2.8$ Hz, H-5').

1. A method of preparing a compound of formula (I)



wherein

n is 0 or 1;

m is 0 or 1;

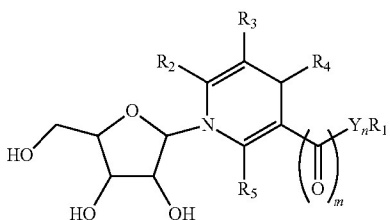
Y is O or S;

R_1 is selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted primary or secondary amino, and substituted or unsubstituted azido;

R_2 - R_5 , which may be the same or different, are each independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, and substituted or unsubstituted aryl; and

X^- is an anion, selected from an anion of a substituted or unsubstituted carboxylic acid, a halide, a substituted or unsubstituted sulfonate, a substituted or unsubstituted phosphate, a substituted or unsubstituted sulfate, a substituted or unsubstituted carbonate, and a substituted or unsubstituted carbamate;

comprising reacting a compound of formula (II)



wherein n , m , Y and R_1 - R_5 are as defined above;

with a compound of the formula Z^+X^- ,

wherein X^- is as defined above, and

wherein Z^+ is a N-containing cation;

in the presence of an aqueous solution and a carbon-containing catalyst;

to form the compound of formula (I).

2. A method according to claim 1, wherein Z^+ is selected from a substituted or unsubstituted ammonium, a substituted or unsubstituted pyridinium, a substituted or unsubstituted

pyrrolidinium, a substituted or unsubstituted imidazolium and a substituted or unsubstituted triazolium.

3. A method according to claim 1 or claim 2, wherein Z^+ is a substituted or unsubstituted ammonium of the formula $N^+HR^I R^I R^I$, wherein R^I , R^I and R^I , which may be the same or different, are each independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, and substituted or unsubstituted aryl.

4. A method according to any one of claims 1 to 3, wherein Z^+ is an unsubstituted ammonium of the formula NH_4^+ .

5. A method according to any preceding claim, wherein the aqueous solution comprises, in addition to water, an organic solvent.

6. A method according to claim 5, wherein the aqueous solution comprises water and organic solvent in a respective ratio by volume of from about 1:5 to about 5:1, optionally from about 1:3 to about 3:1, further optionally from about 1:2 to about 2:1, still further optionally about 1:1.

7. A method according to claim 5 or claim 6, wherein the organic solvent is selected from a substituted or unsubstituted ether, a substituted or unsubstituted ester, a substituted or unsubstituted ketone, a substituted or unsubstituted aliphatic or aromatic hydrocarbon, or a combination thereof.

8. A method according to any one of claims 5 to 7, wherein the organic solvent comprises an ether selected from diethyl ether, methyl tert-butyl ether, ethyl tert-butyl ether, di-tert-butyl ether, diisopropyl ether, dimethoxymethane, tetrahydrofuran, 2-methyltetrahydrofuran, and tetrahydropyran, or a combination thereof.

9. A method according to any one of claims 5 to 8, wherein the organic solvent comprises an ester selected from methyl acetate, ethyl acetate, isopropyl acetate, n-propyl acetate, isobutyl acetate and n-butyl acetate, or a combination thereof.

10. A method according to any one of claims 5 to 9, wherein the organic solvent comprises a ketone selected from methyl isobutyl ketone and methyl isopropyl ketone, or a combination thereof.

11. A method according to any one of claims 5 to 10, wherein the organic solvent comprises an unsubstituted aliphatic hydrocarbon solvent selected from pentane, hexane, cyclohexane and heptane, or a combination thereof.

12. A method according to any one of claims 5 to 11, wherein the organic solvent comprises a substituted aliphatic hydrocarbon solvent, optionally a halogenated aliphatic hydrocarbon solvent, further optionally a chlorinated aliphatic hydrocarbon solvent selected from dichloromethane, trichloromethane, tetrachloromethane, 1,2-chloroethane, 1,1,1-trichloroethane and trichloroethylene, or a combination thereof.

13. A method according to any one of claims 5 to 12, wherein the organic solvent comprises an aromatic hydrocarbon solvent selected from benzene, toluene, ethylbenzene and xylene, or a combination thereof.

14. A method according to any preceding claim, wherein the compound of formula (II) and the compound of the formula Z^+X^- are present in a respective molar ratio of from about 1:5 to about 5:1, 35 optionally from about 1:3 to about 3:1, further optionally from about 1:2 to about 2:1, still further optionally about 1:1.

15. A method according to any preceding claim, wherein the compound of formula (II) and the carbon-containing catalyst are present in a respective molar ratio of from about 10:1

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to about 1:10, optionally from about 5:1 to about 1:5, further optionally from about 4:1 to about 1:4, still further optionally about 1:1 or 1:2 or 1:3 or 1:4.

16. A method according to any preceding claim, wherein the reaction is carried out in a pH range of from about 6 to about 8, optionally from about 6.5 to about 7.5.

17. A method according to any preceding claim, wherein the reaction is carried out at a temperature of from about 10° C. to about 40° C., optionally from about 15° C. to about 35° C., further optionally from about 15° C. to about 30° C., still further optionally from about 15° C. to about 20° C., even further optionally from about 20° C. to about 25° C., even further optionally at a temperature of about 20° C. or 21° C. or 22° C. or 23° C. or 24° C. or 25° C.

18. A method according to any preceding claim, wherein the reaction is carried out for a period of time of from about 1 minute to about 180 minutes, optionally from about 2 minutes to about 120 minutes, further optionally from about 5 minutes to about 120 minutes, still further optionally from about 10 minutes to about 120 minutes, even further optionally from about 20 minutes to about 120 minutes, even further optionally from about 30 minutes to about 120 minutes, still further optionally from about 60 minutes to about 120 minutes, even further optionally from about 60 minutes to about 90 minutes, still further optionally about 60 minutes or 70 minutes or 80 minutes.

19. A method according to any preceding claim, wherein X^- is an anion of a substituted or unsubstituted carboxylic acid selected from an anion of a substituted or unsubstituted monocarboxylic acid and an anion of a substituted or unsubstituted dicarboxylic acid.

20. A method according to claim 19, wherein X^- is an anion of a substituted monocarboxylic acid, optionally an anion of a substituted propanoic acid or an anion of a substituted acetic acid.

21. A method according to claim 20, wherein X^- is an anion of a substituted propanoic acid, optionally an anion of a hydroxy propanoic acid, further optionally an anion of 2-hydroxypropanoic acid, being lactate.

22. A method according to claim 20, wherein X^- is an anion of a substituted acetic acid, being a substituted acetate, optionally a trihaloacetate selected from trichloroacetate, tribromoacetate and trifluoroacetate, further optionally trifluoroacetate.

23. A method according to claim 19, wherein X^- is an anion of an unsubstituted monocarboxylic acid selected from formic acid, acetic acid, propionic acid and butyric acid, being formate, acetate, propionate and butyrate, respectively.

24. A method according to claim 19, wherein X^- is an anion of a substituted or unsubstituted amino-monocarboxylic acid or an anion of a substituted or unsubstituted amino-dicarboxylic acid, wherein, optionally, X^- is an anion of an amino-dicarboxylic acid, further optionally selected from glutamic acid and aspartic acid, being glutamate and aspartate, respectively.

25. A method according to claim 19, wherein X^- is an anion of ascorbic acid, being ascorbate.

26. A method according to any one of claims 1 to 18, wherein X^- is a halide selected from chloride, bromide, fluoride and iodide, optionally chloride or bromide.

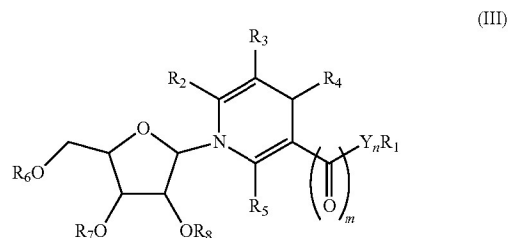
27. A method according to any one of claims 1 to 18, wherein X^- is selected from chloride, acetate, formate, trifluoroacetate, ascorbate, aspartate, glutamate and lactate, optionally selected from chloride, acetate, formate and trifluoroacetate.

28. A method according to any one of claims 1 to 18, wherein the compound of the formula Z^+X^- is selected from ammonium chloride, ammonium acetate, ammonium formate, ammonium trifluoroacetate, ammonium ascorbate, ammonium aspartate, ammonium glutamate and ammonium lactate, optionally selected from ammonium chloride, ammonium acetate, ammonium formate and ammonium trifluoroacetate.

29. A method according to any preceding claim, wherein the method further comprises a filtration step to remove the carbon-containing catalyst from the compound of formula (I).

30. A method according to any one of claims 1 to 18, wherein, in the compound of formula (I), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, and X^- is selected from chloride, acetate, formate and trifluoroacetate.

31. A method according to any preceding claim, wherein the compound of formula (II) is prepared by reacting a compound of formula (III)



wherein

n , m , Y and R_1 - R_5 are as defined in the preceding claims; and

R_6 , R_7 and R_8 , which may be the same or different, are each independently a hydroxyl-protecting group;

with a deprotecting agent;

to form the compound of formula (II).

32. A method according to claim 31, wherein R_6 , R_7 and R_8 are each independently an ester-type protecting group, an ether-type protecting group, or a silyl-type protecting group.

33. A method according to claim 32, wherein an ester-type protecting group is a protecting group selected from acetyl, propionyl, isopropionyl, benzoyl, and trihaloacetyl, optionally trifluoroacetyl or trichloroacetyl.

34. A method according to claim 32, wherein an ether-type protecting group is a protecting group selected from benzyl, p-methoxybenzyl, methoxymethyl and allyl ethers.

35. A method according to claim 32, wherein a silyl-type protecting group is a protecting group selected from trimethylsilyl, triethylsilyl, triisopropylsilyl, 2-(trimethylsilyl)ethoxymethyl, tert-butyldimethylsilyl, tert-butyldiphenylsilyl and tetraisopropylsilyl.

36. A method according to any one of claims 31 to 33, wherein the R_6 , R_7 and R_8 moieties are selected from substituted and unsubstituted acetyl, and substituted and unsubstituted benzoyl.

37. A method according to claim 36, wherein least two of R_6 , R_7 and R_8 are selected from unsubstituted acetyl or unsubstituted benzoyl.

38. A method according to any one of claims 31 to 37, wherein the deprotecting agent is an acid or a base.

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39. A method according to claim 37, wherein R_6 , R_7 and R_8 each comprise unsubstituted acetyl or unsubstituted benzoyl and the deprotecting agent is a base, optionally selected from NH_3 , Na_2CO_3 and $NaOH$.

40. A method according to any one of claims 31 to 39, wherein the reaction is carried out in the presence of a protic or aprotic solvent or a combination thereof.

41. A method according to claim 41, wherein the reaction is carried out in the presence of a protic solvent selected from water, substituted or unsubstituted alcohol, or a combination thereof.

42. A method according to claim 42, wherein the reaction is carried out in the presence of methanol.

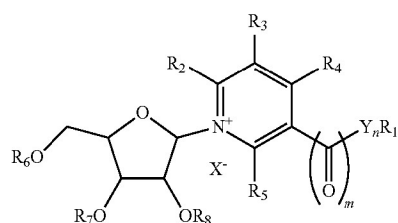
43. A method according to any one of claims 31 to 42, wherein the reactants are subjected to mechanical grinding, optionally using a ball milling or planetary ball milling machine.

44. A method according to any one of claims 31 to 43, wherein, in the compound of formula (III), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, and R_6 - R_8 are each acetyl.

45. A method according to any one of claims 31 to 43, wherein, in the compound of formula (III), n is 1, Y is O, m is 1, R_1 is ethyl, R_2 - R_5 are each H, and R_6 - R_8 are each acetyl.

46. A method according to any one of claims 31 to 43, wherein, in the compound of formula (III), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, and R_6 - R_8 are each benzoyl.

47. A method according to any one of claims 31 to 46, wherein the compound of formula (III) is prepared by reacting a compound of formula (IV)



(IV)

wherein

n , m , Y , R_1 - R_8 and X^- are as defined in the preceding claims;

with a reducing agent,

an aqueous solution,

and an organic solvent,

to form a compound of formula (III).

48. A method according to claim 47, wherein the organic solvent is as defined in any one of claims 7 to 13.

49. A method according to claim 47 or claim 48, wherein when at least two of R_6 , R_7 and R_8 comprise unsubstituted acetyl, the organic solvent is selected from dichloromethane, 1,2-chloroethane, n -butyl acetate, chloroform and ethyl acetate, or a combination thereof, further optionally ethyl acetate.

50. A method according to claim 47 or claim 48, wherein when at least two of R_2 , R_3 and R_4 comprise unsubstituted benzoyl, the organic solvent is selected from trichloroethylene, carbon tetrachloride, diisopropyl ether, toluene, methyl tert-butyl ether, benzene and diethyl ether, or a combination thereof, further optionally diethyl ether.

51. A method according to any one of claims 47 to 50, wherein the reducing agent is selected from sodium dithionite or sodium borohydride.

52. A method according to any one of claims 47 to 51, wherein the method comprises the additional steps of separating the organic phase from the aqueous phase; and extracting the compound of formula (III) from the organic solvent.

53. A method according to any one of claims 47 to 52, wherein the reactants are subjected to mechanical grinding, optionally using a ball mill or planetary ball milling machine.

54. A method according to any one of claims 47 to 53, wherein, in the compound of formula (IV), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, R_6 - R_8 are each acetyl, and X^- is $-OTf$.

55. A method according to any one of claims 47 to 53, wherein, in the compound of formula (IV), n is 1, Y is O, m is 1, R_1 is ethyl, R_2 - R_5 are each H, R_6 - R_8 are each acetyl, and X^- is $-OTf$.

56. A method according to any one of claims 47 to 53, wherein, in the compound of formula (IV), n is 0, m is 1, R_1 is NH_2 , R_2 - R_5 are each H, and R_6 - R_8 are each benzoyl, and X^- is $-OTf$.

* * * * *