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UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

APOTEX INC., a Canadian corporation, Plaintiff, v. GILEAD SCIENCES, INC., a Delaware corporation, and ASTELLAS PHARMA US, INC., a Delaware corporation, Defendants.	Case No. **COMPLAINT FOR DECLARATORY JUDGMENT**

Plaintiff Apotex Inc. ("Apotex"), through counsel, brings this action against Gilead Sciences, Inc. ("Gilead") and Astellas Pharma US, Inc. ("Astellas") (collectively, "Defendants"), for a declaratory judgment that the claims of the patents at issue are not infringed.

INTRODUCTION

1. This is a declaratory judgment action seeking a declaration of non-infringement of U.S. Patent Nos. 8,106,183 ("183 Patent") and 9,085,601 ("601 Patent") (collectively, "Patents-in-Suit") to enable Apotex to bring its generic regadenoson injection, 0.4 mg/5 mL (0.08 mg/mL) prefilled syringe product ("Proposed Regadenoson Product") to market at the earliest possible date under the applicable statutory and regulatory provisions and to allow the public to enjoy the benefits of generic competition for this product.

THE PARTIES

2. Apotex Inc. is a Canadian corporation having its principal place of business at 150 Signet Drive, Toronto, Ontario M9L1T9, Canada.

3. On information and belief, Gilead is a Delaware corporation having its principal place of business at 333 Lakeside Drive, Foster City, California, 94404.

4. On information and belief, Astellas is a Delaware corporation having its principal place of business at 1 Astellas Way, Northbrook, Illinois 60062.

5. On information and belief, Astellas has been registered to do business in California since April 1, 2005 under Entity Number C2740303.

6. On information and belief, Astellas has designated Corporation Service Company Which Will Do Business in California as CSC – Lawyers Incorporating Service, which is located at 2710 Gateway Oaks Drive, Suite 150N, Sacramento, California 95833, to receive service on behalf of Astellas in the State of California.

7. On information and belief, Astellas has represented to the Secretary of State for the State of California that it engages in "Sales & Marketing" in California.

JURISDICTION AND VENUE

8. This Complaint arises under the Patent Laws of the United States, 35 U.S.C. § 100 *et seq.*, the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 302 *et seq.*, as amended by the Drug Price Competition and Patent Term Restoration Act of 1984, Pub. L. No. 98-417, 98 Stat. 1585 (1984 (codified as amended at 21 U.S.C. § 355)) ("Hatch-Waxman Amendments"), and the Medicare Prescription Drug, Improvement, and Modernization Act of 2003, Pub. L. No. 108-173, 117 Stat. 2066 (2003) ("MMA"), based upon an actual controversy between the parties to declare that Apotex is free, upon approval by the Food and Drug Administration ("FDA"), to manufacture, use, market, sell, offer to sell, and/or import the Proposed Regadenoson Product as described in Abbreviated New Drug Application ("ANDA") No. 207604 upon a finding that Apotex does not infringe the Patents-in-Suit.

9. This Court has original jurisdiction over the subject matter of these claims pursuant to 28 U.S.C. §§ 1331 and 1338(a).

10. This Court has personal jurisdiction over Gilead because Gilead's principal place of business is located at 333 Lakeside Drive, Foster City, California, 94404.

11. This Court has personal jurisdiction over Gilead because Gilead, upon information and belief, directly or indirectly markets and sells pharmaceutical products throughout the United States and in this judicial district. Upon information and belief, Gilead purposefully has conducted and continues to conduct business in this judicial district, and this judicial district is a destination of Gilead's pharmaceutical products. Upon information and belief, Gilead has previously submitted to the jurisdiction of this Court and has further previously availed itself of this Court by filing suit in this jurisdiction. *See, e.g.*, *Gilead Sciences, Inc. v. Merck & Co., Inc. et al.*, 13-cv-04057-BLF (N.D. Cal.).

12. On information and belief, Astellas has made sales of pharmaceutical products to an Astellas "Authorized Distributor of Record" in the State of California, including at least to McKesson Corporation, located at One Post Street, San Francisco, California 94104.

13. This Court has personal jurisdiction over Astellas because Astellas has designated an agent in the State of California for service of process and has represented to the Secretary of State for the State of California that it engages in "Sales & Marketing" in California.

14. This Court has personal jurisdiction over Astellas because Astellas has continuous and systematic contacts with the State of California, including the conducting of substantial and regular business activities throughout the State, including the sales and marketing of its pharmaceutical products, including but not limited to LEXISCAN (regadenoson) Injection, 0.4 mg/5 mL (0.08 mg/mL) Prefilled Syringe.

15. This Court has personal jurisdiction over Astellas because Astellas, upon information and belief, directly or indirectly markets and sells pharmaceutical products throughout the United States and in this judicial district. Upon information and belief, Astellas purposefully has conducted and continues to conduct business in this judicial district, and this judicial district is a destination of Astellas' pharmaceutical products, including but not limited to LEXISCAN (regadenoson) Injection, 0.4 mg/5 mL (0.08 mg/mL) Prefilled Syringe.

16. Venue is proper in this District under 28 U.S.C. §§ 1391(b), (c), 1400(b), and/or 21 U.S.C. § 355.

PATENTS-IN-SUIT

17. On its face, the 183 Patent is titled "PROCESS FOR PREPARING AN A_{2A}-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS," and indicates it was issued by the United States Patent and Trademark Office ("PTO")

on January 31, 2012. A true and correct copy of the 183 Patent is attached as Exhibit A.

18. The 183 Patent, as corrected by the November 24, 2015 Certificate of Correction, lists Jeff Zablocki, Elfatih Elzein, Robert Seemayer, and Travis Lemons as the purported named Inventors.

19. According to the face of the 183 Patent and the PTO's online records, Gilead Sciences, Inc. is the assignee of the 183 Patent.

20. On its face, the 601 Patent is titled "PROCESS FOR PREPARING AN A2A-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS," and indicates it was issued by the PTO on July 21, 2015. A true and correct copy of the 601 Patent is attached as Exhibit B.

21. The 601 Patent lists Jeff Zablocki, Elfatih Elzein, Robert Seemayer, and Travis Lemons as the purported named Inventors.

22. According to the face of the 601 Patent and the PTO's online records, Gilead Sciences, Inc. is the assignee of the 601 Patent.

STATUTORY BACKGROUND

23. Under the Hatch-Waxman Amendments, when a company files a New Drug Application ("NDA"), it must, *inter alia*, identify those patents "with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug." 21 U.S.C. § 355(b)(1). Then, as required by statute, after an NDA is approved, FDA publishes the enumerated patents in a publication entitled *Approved Drug Products with Therapeutic Equivalence Evaluations* (colloquially, "Orange Book"). *Id.*

24. Under this same statutory scheme, when a company files an ANDA, the Hatch-Waxman Amendments require, *inter alia*, that an ANDA applicant submit a patent certification with respect to each patent listed for that particular product in the Orange Book at the time the ANDA application is filed. 21 U.S.C.

§ 355(j)(2)(A)(vii).

25. While there are four certification options from which an ANDA applicant can choose, the only certification relevant to this action is a certification that an Orange Book-listed patent "is invalid or will not be infringed by the manufacture, use, or sale of the new drug for which the application is submitted"—a so-called "Paragraph IV Certification." 21 U.S.C. § 355(j)(2)(A)(vii)(IV).

26. As an incentive to promote the filing of ANDAs with Paragraph IV Certifications (and the resulting challenge of Orange Book-listed patents), the Hatch-Waxman Amendments provide that the first ANDA applicant to file an ANDA containing a Paragraph IV Certification for a particular drug product ("First Filer") will receive 180 days of commercial marketing exclusivity for that drug product prior to FDA approving any subsequently filed ANDAs. 21 U.S.C. § 355(j)(5)(B)(iv).

27. If an ANDA applicant elects to file a Paragraph IV Certification to one or more of the Orange Book-listed patents, it is required by statute to provide written notice of the certification to (1) the owner of each patent that is the subject of the certification and (2) the holder of the approved NDA application—a so-called "Paragraph IV Notice Letter." 21 U.S.C. § 355(j)(2)(B).

28. In December 2003, Congress passed the MMA which, *inter alia*, incorporated changes to the Hatch-Waxman Amendments. Title XI of the MMA, *Access to Affordable Pharmaceuticals*, included a provision that allows an ANDA applicant who has not been sued within forty-five days of the NDA holder or patent holder's receipt of a Paragraph IV Notice Letter to file a declaratory judgment action seeking a ruling of non-infringement and/or invalidity of any Orange Book-listed patent for which the ANDA applicant submitted a Paragraph IV Certification. 21 U.S.C. § 355(j)(5)(C).

29. While the First Filer has always been *eligible* for 180 days of

marketing exclusivity under the Hatch-Waxman Amendments, this exclusivity is not absolute. In 2003, the MMA added six express statutory forfeiture provisions that allow for an applicant otherwise entitled to this exclusivity to forfeit the exclusivity if certain conditions are met. 21 U.S.C. § 355(j)(5)(D)(i).

30. One such forfeiture provision (commonly referred to as the "failure to market" provision) requires, *inter alia*, the entry of a judgment of non-infringement and/or invalidity with respect to the patents against which the first ANDA applicant has filed (and maintained) a Paragraph IV Certification, regardless of whether those patents are, or have been in the past, asserted against any subsequent ANDA applicants. 21 U.S.C. § 355(j)(5)(D)(i)(I)(bb).

31. Specifically, the statute recites that the failure to market provision can be triggered by, *inter alia*, "a final decision from which no appeal . . . has been or can be taken that the patent is invalid or not infringed." 21 U.S.C. § 355(j)(5)(D)(i)(I)(bb)(AA).

32. If a subsequent ANDA applicant who has obtained such a judgment has also received Tentative Approval of its ANDA, the First Filer has 75 days after the date of that judgment in which to commercially launch its product, otherwise, the First Filer's 180-day exclusivity is forfeited. 21 U.S.C. § 355(j)(5)(D)(i)(I)(bb).

33. Thus, Congress has *expressly authorized*, and permits, ANDA applicants to bring a declaratory judgment suit for non-infringement and/or invalidity for the purpose of obtaining a court decision sufficient to not only give patent certainty, but also sufficient to cause a forfeiture of the First Filer's 180-day exclusivity under the failure to market forfeiture provision. *See, e.g.*, *Apotex, Inc. v. Daiichi Sankyo, Inc.*, 781 F.3d 1356, 1368-69 (Fed. Cir. 2015) (holding the statute authorizes a subsequent ANDA applicant to file a declaratory judgment action to trigger the 75-day statutory period under the failure to market provision

(21 U.S.C. § 355(j)(5)(D)(i)(I)) that could ultimately result in a forfeiture of the 180-day exclusivity eligibility for the First Filer).

FACTS GIVING RISE TO THIS ACTION

34. On information and belief, Astellas is the current holder of NDA No. 22-161 for LEXISCAN (regadenoson) Injection, 0.4 mg/5 mL (0.08 mg/mL) Prefilled Syringe.

35. On information and belief, Astellas has, as required by statute, identified certain patents for listing in the Orange Book entry for LEXISCAN (regadenoson) Injection, 0.4 mg/5 mL (0.08 mg/mL) Prefilled Syringe.

36. The Patents-in-Suit are among the patents listed in the Orange Book as covering Astellas' LEXISCAN product.

37. On information and belief, by submitting the Patents-in-Suit for listing in the Orange Book, Astellas believed (and continues to believe) the Patents-in-Suit are patents for which "a claim of patent infringement could reasonably be asserted if a person not licensed by the owner engaged in the manufacture, use, or sale of the drug" with respect to its LEXISCAN product. 21 U.S.C. § 355(b)(1).

38. The Patents-in-Suit remain listed in the Orange Book for NDA No. 22-161, and Astellas maintains and continues to represent to the public that the Patents-in-Suit continue to be patents for which a claim of patent infringement could reasonably be asserted against any unlicensed ANDA applicant who attempts to market a generic version of Astellas' LEXISCAN product prior to the expiration of the Patents-in-Suit.

39. According to the Orange Book listing for NDA No. 22-161, the Patents-in-Suit together claim to protect the drug substance (regadenoson) and drug product (LEXISCAN).

40. On information and belief, the 183 Patent was submitted to FDA for listing in the Orange Book on March 8, 2012.

41. On information and belief, on April 10, 2012, an ANDA applicant other than Apotex submitted the first Paragraph IV Certification to FDA challenging a patent listed in the Orange Book for LEXISCAN.

42. Subsequent to April 10, 2012, Apotex filed its ANDA seeking FDA approval for the commercial manufacture, use, importation, offer for sale and sale of the Proposed Regadenoson Product.

43. Astellas' affirmative act of submitting the Patents-in-Suit for listing in the Orange Book created market entry barriers that Apotex, through this action, seeks to eliminate.

44. As part of its ANDA, Apotex filed Paragraph IV Certifications under 21 U.S.C. § 355(j)(2)(A)(vii)(IV) certifying that the Patents-in-Suit will not be infringed by the manufacture, use, or sale of the Proposed Regadenoson Product.

45. In compliance with 21 U.S.C. § 355(j)(2)(B), Apotex sent its Paragraph IV Notice Letter to Gilead and Astellas informing Defendants that Apotex's ANDA seeks approval to engage in the commercial manufacture, use, importation, offer for sale, or sale of the Proposed Regadenoson Product prior to the expiration of the Patents-in-Suit.

46. Apotex's Paragraph IV Notice Letter included non-infringement defenses, and contained an Offer of Confidential Access ("OCA"), as required by 21 U.S.C. § 355(j)(5)(C)(i)(III).

47. Apotex's Paragraph IV Notice Letter was received by Gilead by September 4, 2018.

48. Apotex's Paragraph IV Notice Letter was received by Astellas by September 4, 2018.

49. More than forty-five days have elapsed since Apotex's Paragraph IV Notice Letter was received by both Gilead and Astellas.

50. Apotex desires to bring the Proposed Regadenoson Product to market

and to allow the public to enjoy the benefits of full generic competition for these products at the earliest possible date under the applicable statutory and regulatory provisions.

51. On information and belief, absent a final court decision, the earliest possible date that Apotex currently can obtain final FDA marketing approval for the Proposed Regadenoson Product is the earlier of 180 days after the First Filer begins commercial marketing of its approved generic product or February 2, 2027.

52. To date, the first applicant has not indicated when, or even if, it will begin commercial marketing of its approved generic product.

53. On information and belief, neither Astellas nor Gilead has ever filed a complaint alleging infringement of any of the Orange Book patents listed for LEXISCAN, including the Patents-in-Suit.

54. On information and belief, despite having submitted its ANDA more than 78 months ago, the first ANDA applicant has not secured FDA approval and thus has not yet begun commercial marketing its approved regadenoson ANDA product.

55. This failure to obtain approval effectively has blocked competitors from entering the market unless, through a declaratory judgment, a competitor can obtain forfeiture of the First Filer's 180-day exclusivity, as Apotex seeks to do in this action.

56. On information and belief, as is its policy, FDA has not made a determination as to whether the First Filer has forfeited exclusivity under any provision listed in 21 U.S.C. § 355(j)(5)(D)(i) and as a result, the First Filer's presumptive 180-day exclusivity period remains in force.

57. Under the current facts, FDA could be prohibited from granting final approval to the Proposed Regadenoson Product until at least 180 days after the first commercial marketing of the First Filer's approved generic regadenoson injection,

0.4 mg/5 mL (0.08 mg/mL) prefilled syringe product.

58. The Federal Circuit has decided that Tentative Approval is not necessary to adjudicate the requested declaratory judgments. *Apotex*, 781 F.3d at 1364 ("[I]s the prospect of concrete relief for Apotex too uncertain to support an adjudication of the request for a [declaratory] judgment until Apotex obtains tentative approval? We conclude that the answer is no.")

59. Any prohibition on FDA approval that has been created by the First Filer's 180-day exclusivity constitutes an entry barrier for Apotex.

60. Astellas is responsible for the existence of this entry barrier because, upon information and belief, one of the Patents-in-Suit supports the First Filer's exclusivity, and Astellas submitted the Patents-in-Suit for listing in the Orange Book.

61. If Apotex is able to obtain a judgment that the Patents-in-Suit are "invalid or not infringed," as well as receive Tentative Approval for the Proposed Regadenoson Product, the First Filer would have 75 days after the date of that judgment to launch its product. If the First Filer did not launch its product within that 75 day window, it would forfeit its 180-day exclusivity, permitting other generics, such as Apotex, to enter the marketplace earlier than they otherwise would be permitted. 21 U.S.C. § 355(j)(5)(D)(i)(I)(bb).

62. The elimination, truncation, or preservation of the First Filer's presumptive 180-day exclusivity period (including whether Apotex can obtain the requisite judgment regarding that period) has created concrete stakes over which Apotex and Defendants have adverse interests.

63. A decision by this Court in Apotex's favor on its non-infringement claims would remove an entry barrier, the First Filer's exclusivity period, currently prohibiting Apotex from entering the market.

COUNT I

Declaratory Judgment of Non-Infringement of the 183 Patent

64. Apotex re-alleges and incorporates by reference the allegations of paragraphs 1-63 as though fully set forth herein.

65. There is a present, genuine, and justiciable controversy between Apotex and Defendants regarding, *inter alia*, whether the manufacture, use, offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would infringe any valid or enforceable claim of the 183 Patent, either directly or indirectly, that is of sufficient immediacy and reality to warrant the issuance of a Declaratory Judgment.

66. There are substantial, concrete stakes at issue between the parties concerning whether Apotex secures the non-infringement judgment it seeks, as securing such a judgment would result in Apotex advancing its entry into the market.

67. But for Astellas' decision to list the 183 patent in the Orange Book for LEXISCAN, FDA approval of the Proposed Regadenoson Product would not be independently delayed by that patent or any First Filer's exclusivity associated with that patent.

68. As a result, but for Astellas' decision, Apotex would not be subject to this independent entry barrier that Astellas is responsible for creating. Apotex is being injured by Astellas' decision to submit the 183 Patent for listing in the Orange Book and Astellas' continued maintenance of this listing.

69. Apotex's injury can be redressed by the requested relief: a declaratory judgment of non-infringement is necessary to cause forfeiture of the first applicant's exclusivity period, which otherwise will block final marketing approval of the Proposed Regadenoson Product. If Apotex remains blocked by a First Filer's exclusivity, Apotex will be monetarily harmed, as it will lose sales of the

Proposed Regadenoson Product by virtue of not being able to enter the market at the earliest possible date under the applicable statutory and regulatory provisions, and be deprived of an economic opportunity to compete in the market for regadenoson products.

70. The manufacture, use, offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would not infringe any valid or enforceable claim of the 183 Patent, either directly or indirectly.

71. Apotex is entitled to a judicial declaration that the manufacture, use, offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would not infringe, directly or indirectly, any valid or enforceable claim of the 183 Patent, either literally or under the doctrine of equivalents.

COUNT II

Declaratory Judgment of Non-Infringement of the 601 Patent

72. Apotex re-alleges and incorporates by reference the allegations of paragraphs 1-71 as though fully set forth herein.

73. There is a present, genuine, and justiciable controversy between Apotex and Defendants regarding, *inter alia*, whether the manufacture, use, offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would infringe any valid or enforceable claim of the 601 Patent, either directly or indirectly, that is of sufficient immediacy and reality to warrant the issuance of a Declaratory Judgment.

74. There are substantial, concrete stakes at issue between the parties concerning whether Apotex secures the non-infringement judgment it seeks.

75. The manufacture, use, offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would not infringe any valid or enforceable claim of the 601 Patent, either directly or indirectly.

76. Apotex is entitled to a judicial declaration that the manufacture, use,

offer for sale, sale, importation, and/or marketing of the Proposed Regadenoson Product would not infringe, directly or indirectly, any valid or enforceable claim of the 601 Patent, either literally or under the doctrine of equivalents.

PRAYER FOR RELIEF

WHEREFORE, Apotex respectfully requests the Court to enter judgment as follows:

(A) Declaring that the manufacture, use, sale, offer for sale, importation, and/or marketing of the Proposed Regadenoson Product has not infringed, does not infringe, and would not—if made used, sold, offered for sale, imported, or marketed—infringe, either directly or indirectly, any valid and/or enforceable claim of the Patents-in-Suit, either literally or under the doctrine of equivalents;

(B) Declaring that FDA may approve the Proposed Regadenoson Product whenever that application is otherwise in condition for approval, without awaiting any further order, judgment, or decree of this Court; that the judgment entered in this case is a judgment reflecting a decision that the Patents-in-Suit are not infringed pursuant to 21 U.S.C. § 355(j)(5)(D)(i)(I)(bb);

(C) Declaring this case exceptional and awarding Apotex its reasonable attorneys' fees and costs under 35 U.S.C. § 285; and

(D) Awarding Apotex such other and further relief as the Court may deem just and proper.

Dated: October 23, 2018

Respectfully Submitted,

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Exhibit A

US008106183B2

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

(10) **Patent No.: US 8,106,183 B2**
(45) **Date of Patent: *Jan. 31, 2012**

(54) **PROCESS FOR PREPARING AN A_{2A}-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS**

(75) Inventors: **Jeff Zablocki**, Mountain View, CA (US); **Elfaith Elzein**, Fremont, CA (US)

(73) Assignee: **Gilead Sciences, Inc.**, Foster City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/765,623**

(22) Filed: **Apr. 22, 2010**

(65) **Prior Publication Data**

US 2010/0267953 A1 Oct. 21, 2010

Related U.S. Application Data

(63) Continuation of application No. 11/701,699, filed on Feb. 2, 2007, now Pat. No. 7,732,595.

(60) Provisional application No. 60/801,857, filed on May 18, 2006, provisional application No. 60/765,114, filed on Feb. 3, 2006.

(51) **Int. Cl.**
C07H 19/167 (2006.01)

(52) **U.S. Cl.** **536/27.11**; 536/27.61

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Disclosed is a synthesis suitable for large scale manufacture of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

9 Claims, 5 Drawing Sheets

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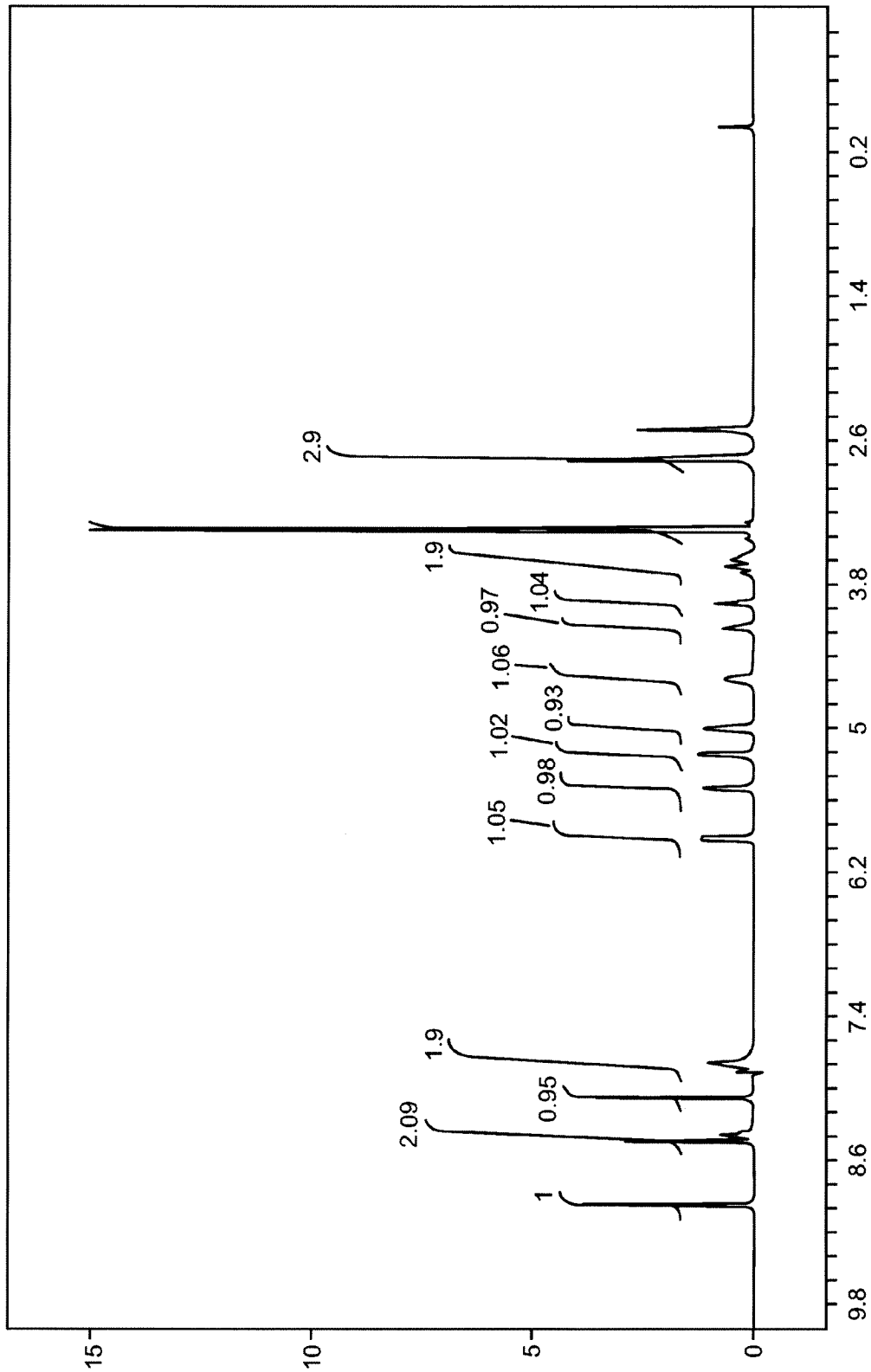
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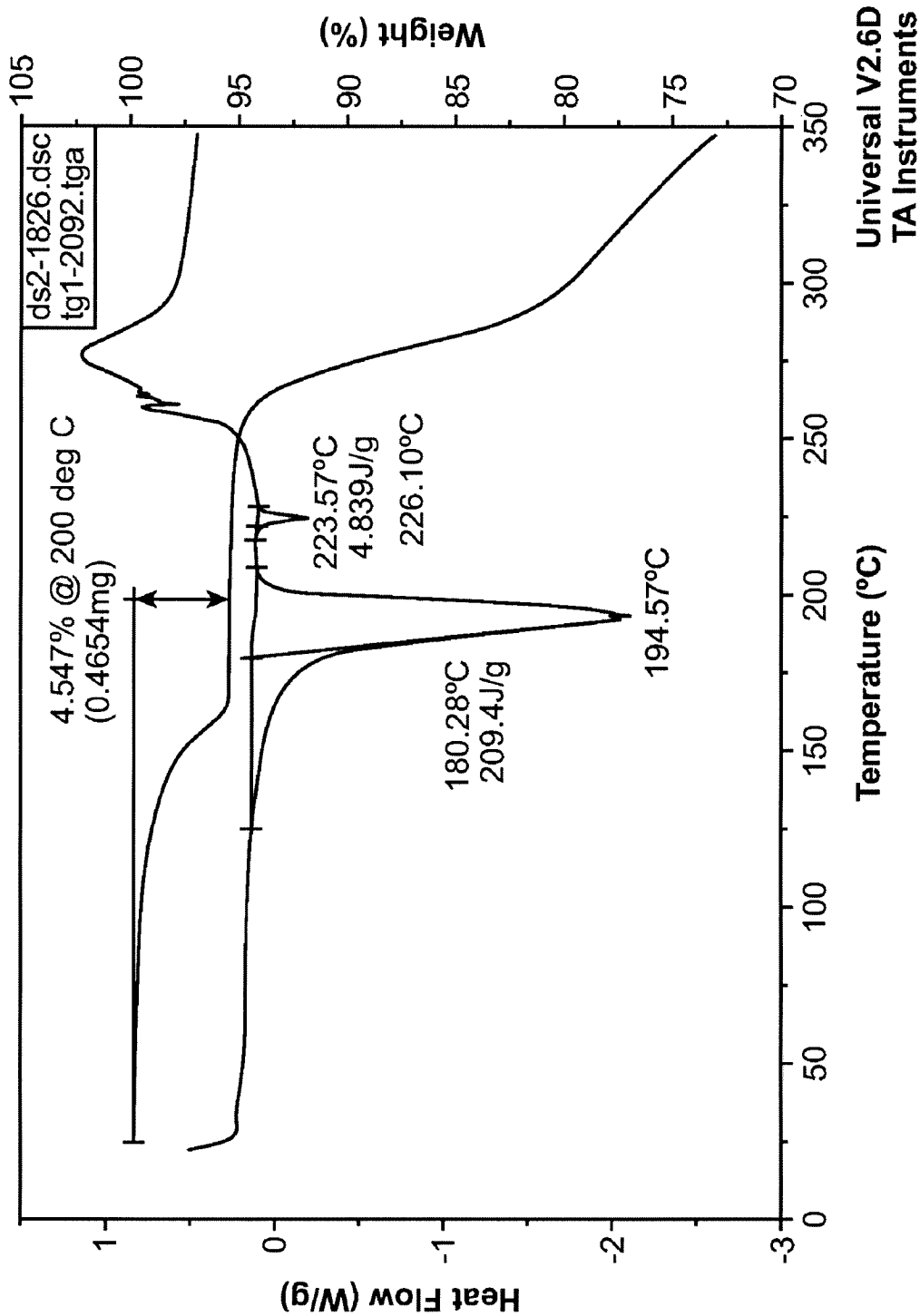
FIG. 1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209.4J/g

FIG. 2

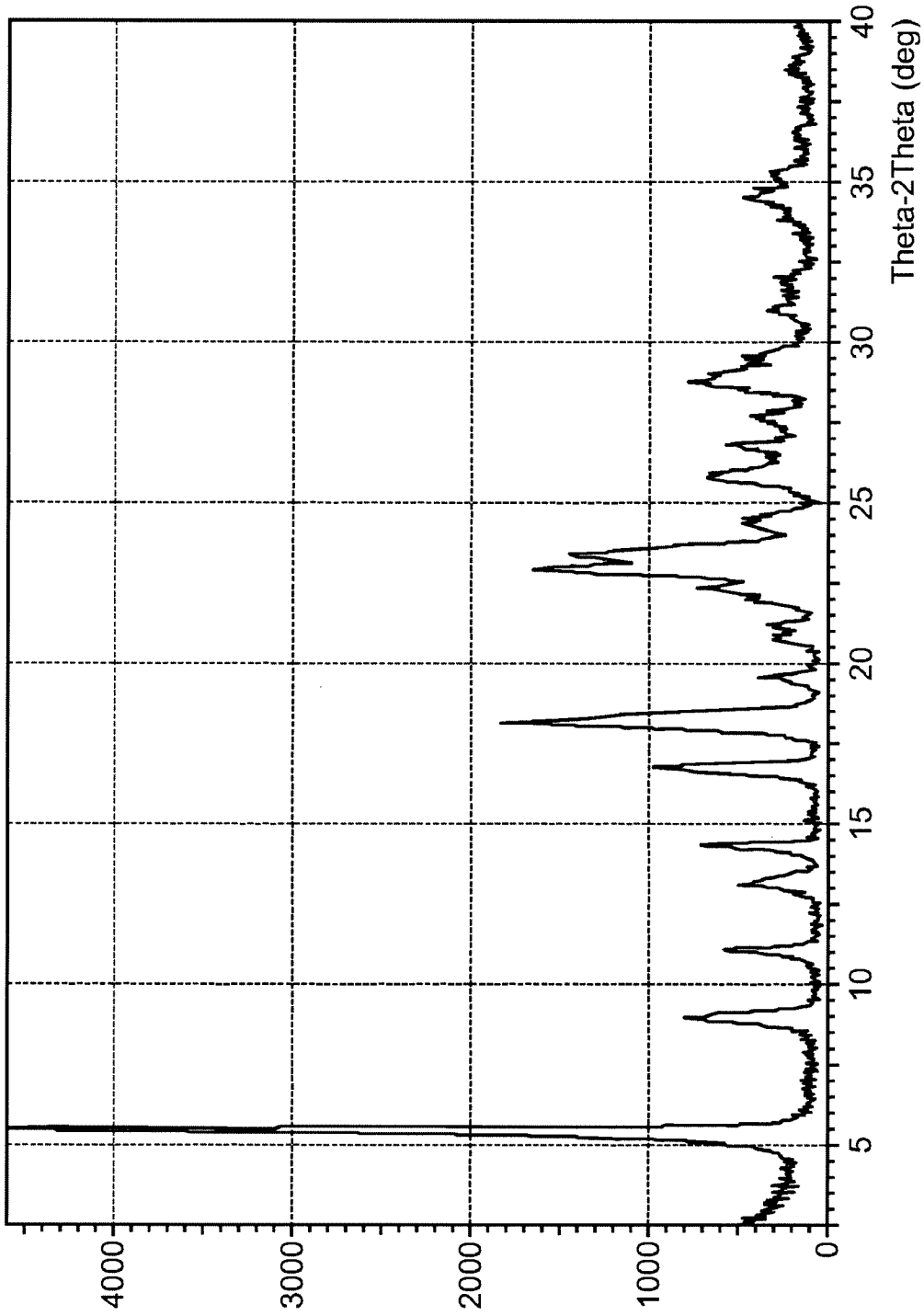

FIG. 3

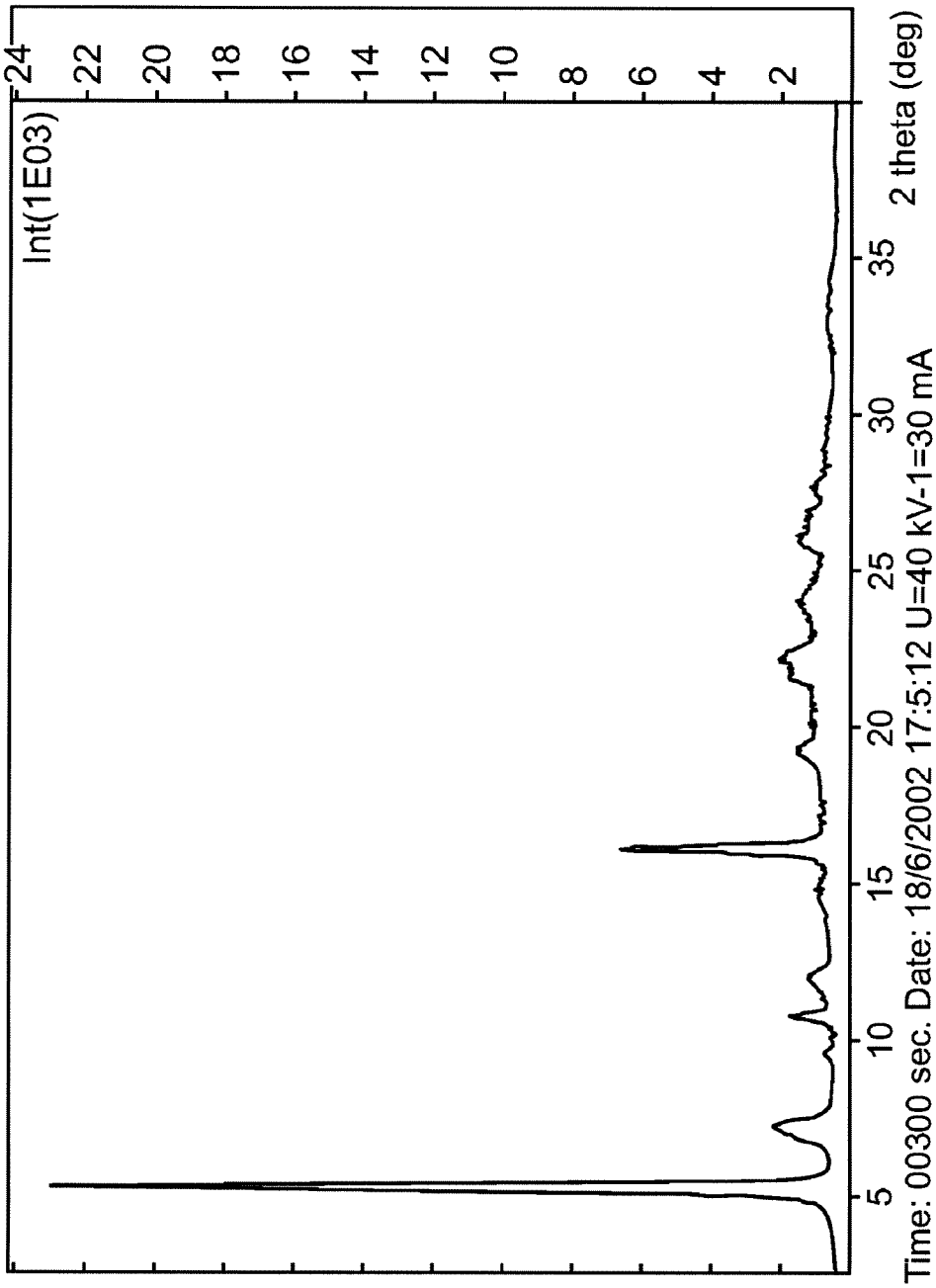
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FIG. 4

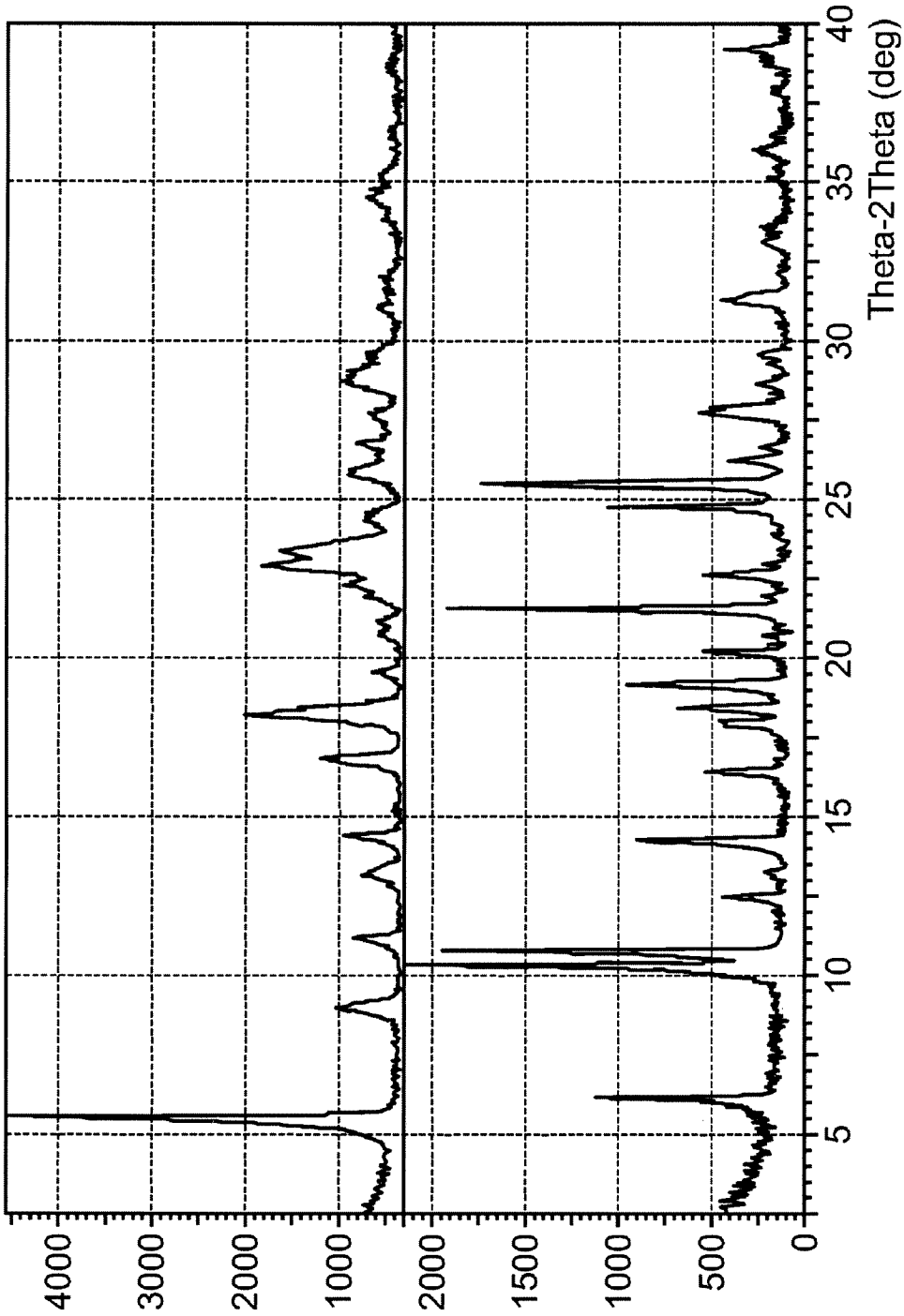
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FIG. 5

PROCESS FOR PREPARING AN A_{2A}-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 11/701,699, filed Feb. 2, 2007, now issued as U.S. Pat. No. 7,732,595, which claims priority to U.S. Provisional Patent Application Ser. No. 60/801,857, filed May 18, 2006, and to U.S. Provisional Patent Application Ser. No. 60/765,114, filed Feb. 3, 2006, which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to a process for the large scale preparation of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

BACKGROUND

Adenosine is a naturally occurring nucleoside, which exerts its biological effects by interacting with a family of adenosine receptors known as A_1, A_{2A}, A_{2B}, and A_3, all of which modulate important physiological processes. One of the biological effects of adenosine is to act as a coronary vasodilator; this result being produced by interaction with the A_{2A} adenosine receptor. This effect of adenosine has been found to be useful as an aid to imaging of the heart, where coronary arteries are dilated prior to administration of an imaging agent (for example thallium 201), and thus, by observation of the images thus produced, the presence or absence of coronary artery disease can be determined. The advantage of such a technique is that it avoids the more traditional method of inducing coronary vasodilation by exercise on a treadmill, which is clearly undesirable for a patient that has a coronary disease.

However, administration of adenosine has several disadvantages. Adenosine has a very short half life in humans (less than 10 seconds), and also has all of the effects associated with A_1, A_{2A}, A_{2B}, and A_3 receptor agonism. Thus the use of a selective A_{2A} adenosine receptor agonist would provide a superior method of producing coronary vasodilation, particularly one with a longer half life and few or no side effects.

A class of compounds possessing these desirable properties was disclosed in U.S. Pat. No. 6,403,567, the complete disclosure of which is hereby incorporated by reference. In particular, one compound disclosed in this patent, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, has been shown to be a highly selective A_{2A}-adenosine receptor agonist, and is presently undergoing clinical trials as a coronary vasodilator useful in cardiac imaging.

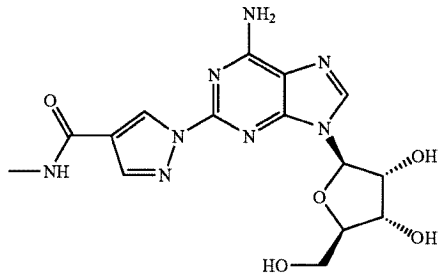
Given the heightened interest in this and similar compounds, it has become desirable to find new methods of synthesis that provide a convenient method for making large quantities of the material in good yield and high purity. The patent that discloses the compound of interest (U.S. Pat. No. 6,403,567) provides several methods for preparing the compound. However, although these methods are suited to small scale syntheses, all synthetic methods disclosed in the patent utilize protecting groups, which is undesirable for large scale syntheses.

Additionally, it was discovered that the desired product (that is (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide) is capable of existing in at least three different crystalline forms, the most stable of which is a monohydrate. This polymorph is stable under relative humidity stress conditions, up to its melting point. Accordingly, it is desirable that the final product produced in the new syntheses is obtained as the stable monohydrate.

SUMMARY OF THE INVENTION

Thus, it is an object of this invention to provide convenient syntheses for the large scale preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, and polymorphs thereof, preferably as its monohydrate. Accordingly, in a first aspect, the invention relates to the preparation of a compound of the Formula I:

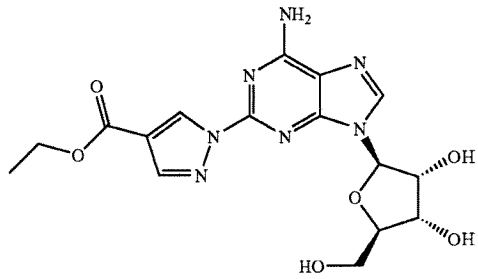
Formula I



comprising:

contacting a compound of the formula (3):

(3)



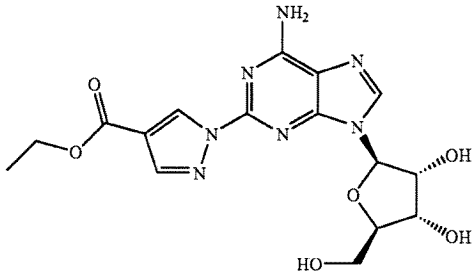
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature of about 0-5° C., followed by warming to about 50-70° C. Alternatively, the reaction is conducted as above but in a sealed pressure reactor.

In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

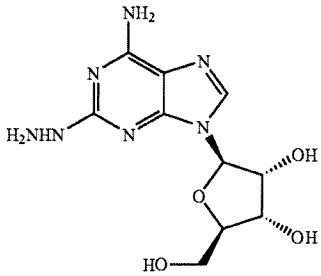
In a second aspect, the invention relates to the preparation of a compound of the formula (3):

(3)



comprising:

contacting a compound of the formula (2):

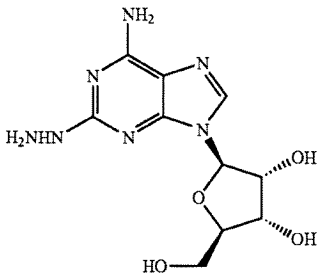


with ethyl 2-formyl-3-oxopropionate.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C., with about 1.1 molar equivalents of ethyl 2-formyl-3-oxopropionate.

In a third aspect, the invention relates to the preparation of a compound of the formula (2):

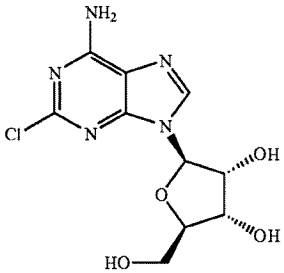
(2)



comprising:

contacting a compound of the formula (1):

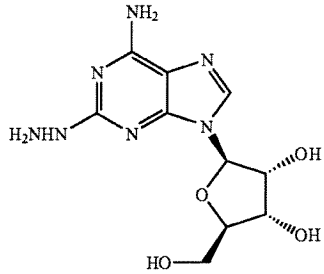
(1)



with hydrazine.

The above described synthesis is suitable for the large scale synthesis of the desired product, which is provided in good yield, although one minor impurity is seen in the final product. This impurity has been shown to be unchanged intermediate of the formula (2); that is, the compound of the formula:

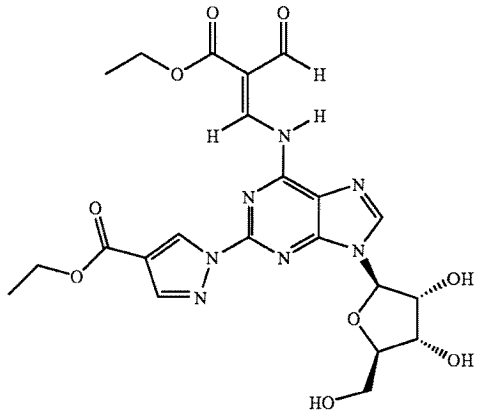
(2)



Although this impurity can be removed from the final product by crystallization, it was decided to seek an alternative synthesis that had all of the advantages of the above synthesis but did not give the compound of formula (2) as an impurity in the final product.

Thus, in a fourth aspect, the invention relates to a method of synthesizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide by contacting a compound of the formula (4):

(4)



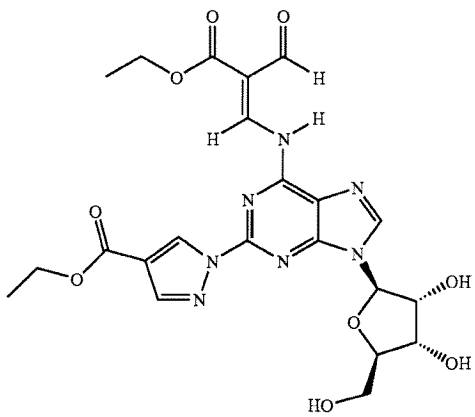
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature of about 0-5° C., followed by warming to about 50-70° C. Preferably, the reaction is conducted in a sealed pressure reactor.

In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

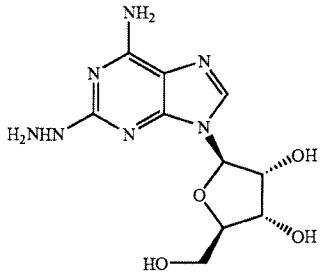
In a fifth aspect, the invention relates to a method of synthesizing a compound of the formula (4):

(4)



comprising contacting a compound of the formula (2):

(2)



with an excess of ethyl 2-formyl-3-oxopropionate, preferably about a 2-10 fold excess, more preferably about a 5-10 fold excess.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C. The ethyl 2-formyl-3-oxopropionate is present in a 5-10 fold excess.

DEFINITIONS AND GENERAL PARAMETERS

FIG. **1** is a ^{1}H NMR spectrum of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (Form A).

FIG. **2** shows the thermal analysis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **3** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **4** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form B.

FIG. **5** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form C as compared to Form A.

As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not.

The term "therapeutically effective amount" refers to that amount of a compound of Formula I that is sufficient to effect treatment, as defined below, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "polymorph" is intended to include amorphous and solvates of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide.

It has been discovered that this compound is capable of existing in at least three different crystalline forms, referred to herein as Form A, Form B, Form C, and an amorphous product.

Form A: This polymorph can be produced by crystallizing 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from protic solvents, for example ethanol or ethanol/water mixtures, or from a polar solvent, for example dimethylsulfoxide/water. Form A has been shown to be a monohydrate, and is the most stable of the various polymorphs at ambient temperatures. It is stable under relative humidity stress conditions up to its melting point.

Form B: This polymorph is produced by evaporating under vacuum a solution of 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in trifluoroethanol at ambient temperatures. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. **4**), but it was difficult to determine its constitution, as the X-ray analysis gave disordered broad peaks, and the polymorph contained varying amounts of water. It was found to be difficult to reliably reproduce the preparation of this polymorph.

Form C: This polymorph is produced by slurrying 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in acetonitrile for a long period of time at 60° C. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. **5**). Polymorph C was shown to be a variable hydrate, which, at elevated temperatures, desolvates to an unstable form.

Amorphous Material: This polymorph is produced by heating Form A polymorph at a temperature of up to 200° C. This

polymorph is unstable in the presence of atmospheric moisture, forming variable hydrates.

Techniques for Analysis of Forms A, B, C and Amorphous Material

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu Kα radiation. The instrument was equipped with a fine focus X-ray tube, and the tube voltage and amperage were set to 40 kV and 40 mA respectively. The divergence and scattering slits were set at 1" and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5-40° 2θ was used. A silicon standard was used to check the instrument alignment. Data were collected and analyzed using XRD-6000 v. 4.1 software.

X-ray powder diffraction (XRPD) analyses were also performed using an Inel XRG-3000 diffractometer equipped with a CPS (Curved Position Sensitive) detector with a 28 range of 120°. The instrument calibration was performed using a silicon reference standard. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The monochromator slit was set at 5 mm by 80 μm. Samples were placed in an aluminum sample holder with a silicon insert or in glass XRPD-quality capillaries. Each capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. Real time data were collected using Cu—Kα radiation at a resolution of 0.03° 2θ. Typically, data were collected over a period of 300 seconds. Only the data points within the range of 2.5-40° 2θ are displayed in the plotted XRPD patterns.

Thermal Analyses

Thermogravimetric (TG) analyses were carried out on a TA Instruments 2050 or 2950 thermogravimetric analyzer. The calibration standards were nickel and Alumel™. Samples were placed in an aluminum sample pan, inserted into the TG furnace, and accurately weighed. The samples were heated in nitrogen at a rate of 10° C./min to either 300 or 350° C. Unless stated otherwise, samples weights were equilibrated at 25° C. in the TGA furnace prior to analysis.

Differential scanning calorimetry (DSC) analyses were carried out on a TA Instruments differential scanning calorimeter 2920. Accurately weighed samples were placed in either crimped pans or hermetically sealed pans that contained a pinhole to allow for pressure release. Each sample was heated under nitrogen at a rate of 10° C./min to either 300 or 350° C. Indium metal was used as the calibration standard. Temperatures were reported at the transition maxima.

Infrared Spectroscopy

Infrared spectra were acquired on Magna 860® Fourier transform infrared (FT-IR) spectrophotometer (Nicolet Instrument Corp.) equipped with an Ever-Glo mid/far IR source, an extended range potassium bromide beamsplitter, and a deuterated triglycine sulfate (DTGS) detector. Unless stated otherwise, a Spectra-Tech, Inc. diffuse reflectance accessory (the Collector™) was used for sampling. Each spectrum represents 256 co-added scans at a spectral resolution of 4 cm^{-1}. Sample preparation for the compound consisted of placing the sample into a microcup and leveling the material with a frosted glass slide. A background data set was acquired with an alignment mirror in place. The spectra represent a ratio of the sample single-beam data set to the background single beam data set. Wavelength calibration of the instrument was performed using polystyrene.

NMR Spectroscopy

Solution phase ^1H NMR spectra of the were acquired at ambient temperature on a Bruker model AM-250 spectrometer operating at 5.87 T (Larmor frequency: ^1H=250 MHz). Time-domain data were acquired using a pulse width of 7.5 ps and an acquisition time of 1.6834 second over a spectral window of 5000 Hz. A total of 16,384 data points were collected. A relaxation delay time of 5 seconds was employed between transients. Each data set typically consisted of 128 coaveraged transients. The spectra were processed utilizing GRAMS132 A1 software, version 6.00. The free induction decay (FID) was zero-filled to four times the number of data points and exponentially multiplied with a line-broadening factor of 0.61 Hz prior to Fourier transformation. The ^1H spectra were internally referenced to tetramethylsilane (0 ppm) that was added as an internal standard.

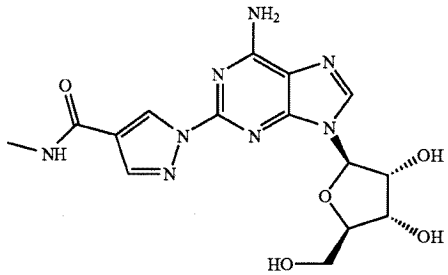
Alternatively, NMR analysis was carried out as described in Example 4.

Moisture SorptionJDesorption Analyses

Moisture sorption/desorption data were collected on a VTI SGA-100 Vapor Sorption Analyzer. Sorption and desorption data were collected over a range of 5% to 95% relative humidity (RK) at 10% RH intervals under a nitrogen purge. Sodium chloride (NaCl) and polyvinyllpyrrolidone (PVP) were used as the calibration standards. Equilibrium criteria used for analysis were less than 0.0100% weight change in 5 minutes, with a maximum equilibration time of 180 minutes if the weight criterion was not met. The plotted data have not been corrected for the initial moisture content.

Nomenclature

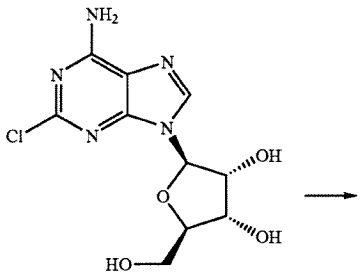
The structure of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is as follows:



Synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide

One method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme I.

REACTION SCHEME I



(1)

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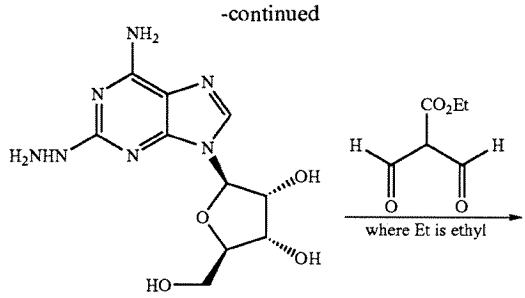
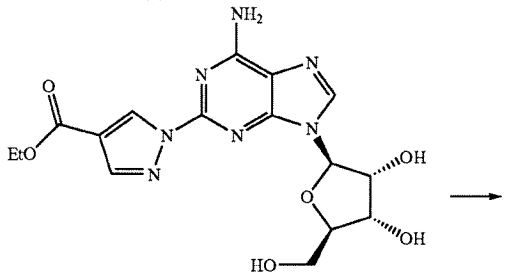
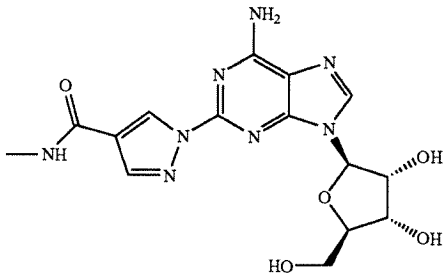


(2)



(3)



Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 40° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (3)

The compound of formula (2) is then converted to a compound of formula (3) by reacting with about 1-1.2 molar equivalents of ethyl 2-formyl-3-oxopropionate. The reaction is conducted in a protic solvent, preferably ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure. The product of formula (3) is taken to the next step without purification.

Step 3—Preparation of Final Product

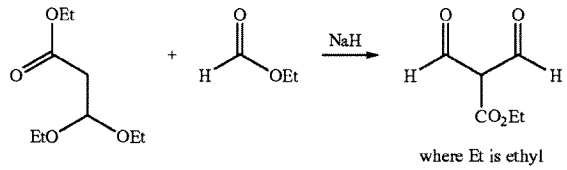
The final product is prepared from the compound of formula (3) by reacting with methylamine, preferably aqueous methylamine. The reaction is carried out at about room temperature, for about 4 hours. The product of Formula I is isolated by conventional means, for example by filtration, washing the solid with cold ethanol, and drying under reduced pressure.

Preparation of Starting Materials

(4S,2R,3R,5R)-2-(6-amino-2-chloropurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol is used as a starting material in step 1. This compound is commercially available.

Ethyl 2-formyl-3-oxopropanoate is used as a starting material in step 2. It is commercially available, or may be made as shown in Reaction Scheme II.

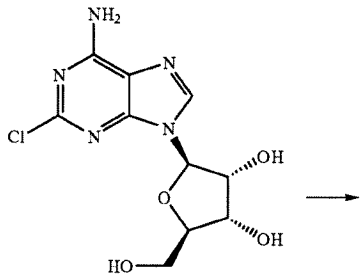
REACTION SCHEME II



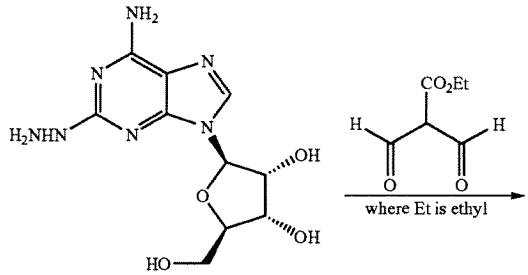
Ethyl 3,3-diethoxypropionate is reacted with ethyl formate in the presence of a strong base, preferably sodium hydride. The reaction is carried out at about 0-5° C., for about 24 hours. The product is isolated by conventional means, for example by the addition of water and extraction of impurities with a conventional solvent, for example t-butylmethyl ether, acidification of the aqueous phase with, for example, hydrochloric acid, followed by extraction with a solvent such as dichloromethane, and removing the solvent from the dried extract under reduced pressure.

A preferred method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme III.

REACTION SCHEME III



(1)



(2)

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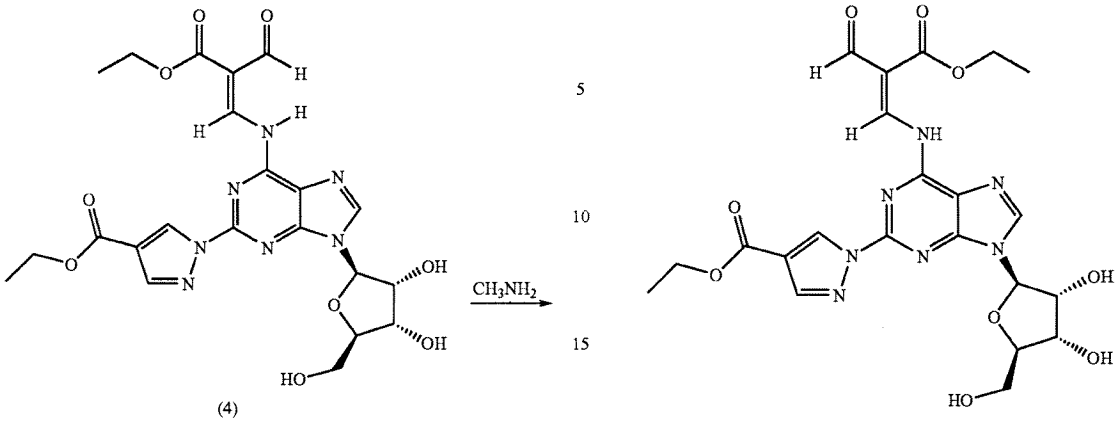
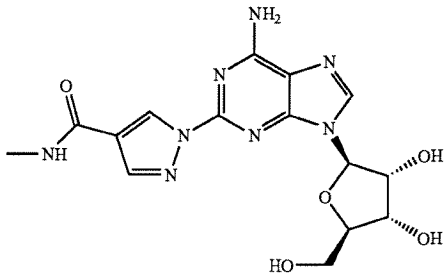


(4)



Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 45-55° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by ethanol or isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (4)

The compound of formula (2) is then converted to a compound of formula (4) by reacting with an excess of ethyl 2-formyl-3-oxopropionate, for example a 2-10 fold excess, preferably about 5-10 fold excess. The reaction is conducted in a protic solvent, for example ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure, and the product of formula (4) is taken to the next step without purification.

The compound of formula (4) is drawn as a (2E) alkene derivative, as this is the major isomer formed in this reaction. However, it should be noted that a significant amount of the (2Z) alkene derivative may also be formed in this reaction; that is:

named as ethyl (2Z)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl) pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

Accordingly, although the compound of formula (4) is represented as the (2E) alkene derivative only, the term "compound of formula (4)" is intended to include both the instance where it is solely the (2E) isomer, and the instance where the major portion of the product is the (2E) isomer and a minor portion of the (2Z) isomer is also present. The conversion of the compound of formula (4) to the final product by reaction with methylamine as described in Step 3 proceeds in the same manner whether the compound of formula (4) is present as the (2E) isomer or as a mixture of the (2E) isomer and the (2Z) isomer.

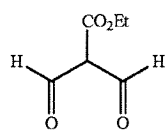
Step 3—Preparation of Final Product

The final product is prepared from the compound of formula (4) by reacting with methylamine, preferably aqueous methylamine. The reaction is initially carried out at about 0-5° C. for about 8 hours, preferably in a pressure reactor, followed by raising the temperature to 50-60° C. over about 1 hour, and maintaining the temperature for 15-30 minutes. The product is isolated by conventional means, for example by cooling to 0-5° C. and maintaining a vacuum for about 1 hour, thus removing the methylamine. The vacuum is removed, and the remaining contents held at 0-5° C. for at least 30 minutes, followed by filtration. The solid thus obtained is washed with water followed by ethanol, and dried under reduced pressure.

This process provides (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate. This polymorph can be further purified by dissolving in dimethylsulfoxide, filtering any solid impurities from the solution, and precipitating the monohydrate from solution by addition of water.

EXAMPLE 1

Preparation of Ethyl-2-formyl-3-oxopropionate



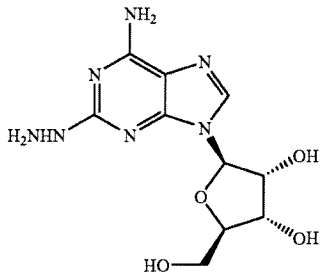
A three- or four-neck round bottom flask equipped with magnetic stir bar, thermocouple, digital thermometer, gas inlet and outlet and addition funnel was flushed with argon.

Ethyl 3,3-diethoxypropionate (64.5 g) in tetrahydrofuran were charged to the addition funnel. Sodium hydride (21.2 g of a 60% dispersion) was charged to the reaction flask followed by tetrahydrofuran. The contents of the flask were cooled to 0-5° C. in an ice-bath, and ethyl formate (257 g) was added. The mixture was cooled to 0-5° C. and the contents of the addition funnel added dropwise, maintaining an internal temperature of less than 5° C. The ice-bath was removed and the contents allowed to warm to ambient temperature. Consumption of ethyl 3,3-diethoxypropionate was monitored by TLC analysis. The reaction was quenched by addition of ice-water (10.6 vol), and extracted three times with methyl t-butyl ether (5.4 vol each), and the organic layers discarded. The aqueous phase was acidified with conc. hydrochloric acid to a pH of 1 to 1.5. The acidified aqueous layer was extracted three times with dichloromethane and the combined organic layers dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue distilled under vacuum, to provide ethyl 2-formyl-3-oxopropionate, 27.92 g, 70% yield.

EXAMPLE 2

A. Preparation of 2-Hydrazinoadenosine (2)

(2)



A flask equipped with a mechanical stirrer, gas inlet, gas outlet and thermocouple was flushed with argon. 2-Chloroadenosine hemihydrate (53.1 g) was added, followed by hydrazine monohydrate (134 g). The mixture was stirred while heating to 40-45° C. for 2 hours. The progress of the reaction was followed by TLC analysis. When the reaction was complete, the heat source was removed and ethanol (800 ml) was added. The mixture was stirred for 2 hours at ambient temperature, then the precipitate collected by filtration. The filter cake was washed with ethanol and dried under reduced pressure for 30 minutes. The solids were transferred to a clean flask equipped with a mechanical stirrer and water (300 ml) was added. The suspension was stirred at room temperature for 18 hours, and the solids isolated by filtration. The filter cake was washed with ice-cold water (300 ml) followed by a wash with ice-cold ethanol (300 ml). The solid was dried under reduced pressure to provide 2-hydrazinoadenosine (41.38 g, 81.4% yield, 99.3% purity).

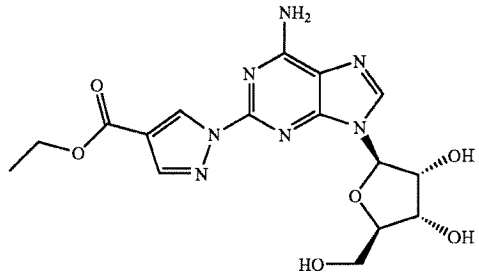
B. Alternative Preparation of 2-Hydrazinoadenosine (2)

A reaction vessel containing hydrazine hydrate (258 g, 250 ml) was heated to 40-50° C. To the warm mixture 2-chloroadenosine hemihydrate (100 g) was added in portions, maintaining the temperature between 45-55° C. The temperature was kept at this temperature for two hours, and then deionized water (500 ml) was added over a period of 30 minutes, maintaining the temperature at 45-55° C. The mixture was then gradually cooled to 0-5° C. over a period of 3 hours, then stirred at this temperature for a further 30 minutes. The solid was then filtered off, and washed with cold (2-5° C.) deionized water (200 ml), followed by ethanol (400 ml). The solid was dried under vacuum for 12 hours, to provide 2-hydrazinoadenosine.

EXAMPLE 3

Preparation of Ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (3)

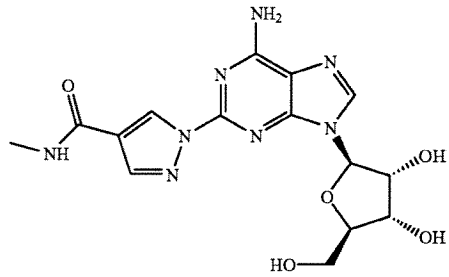
(3)



Ethyl 2-formyl-3-oxopropionate (23.93 g, 0.17 mol) was placed in a flask equipped with mechanical stirrer, gas inlet, gas outlet and reflux condenser. 2-Propanol was added to the flask followed by 2-hydrazinoadenosine (44.45 g, 0.15 mol). The mixture was heated to reflux under stirring for 2-4 hours, following the progress of the reaction by TLC analysis. When the reaction was judged complete, the heat source was removed and the mixture cooled to room temperature. The suspension was cooled under stirring in an ice-bath for 1.5 to 2 hours. The solids were isolated by vacuum filtration, and washed with ice-cold 2-propanol. The product, ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate, was dried under reduced pressure to a constant weight. Yield 54.29 g, purity (by HPLC) 96.6%.

EXAMPLE 4

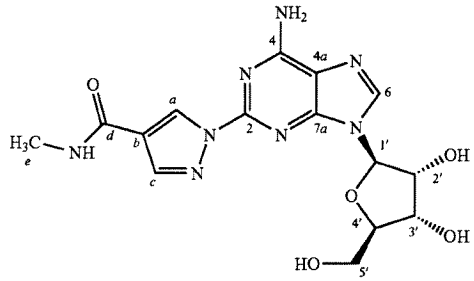
Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide



A mixture of ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (46.4 g) and methylamine (40% in water, 600 ml) was stirred at ambient temperature for about 4 hours, following the progress of the reaction by HPLC analysis. The majority of the excess methylamine was removed under

reduced pressure, and the remaining mixture cooled at 0° C. for 2 hours. The solid material was filtered off, washed with ice-cold 200 proof ethanol, and dried under reduced pressure, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate, 36.6 g, purity 99.6%.

The structure of the material was confirmed by ^{1}H NMR (see FIG. **1** and below). Thermal analysis (see FIG. **2**) provided results consistent with the presence of one molecule of water. X-Ray powder diffraction patterns were obtained (FIG. **3**)



^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6-DMSO—5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

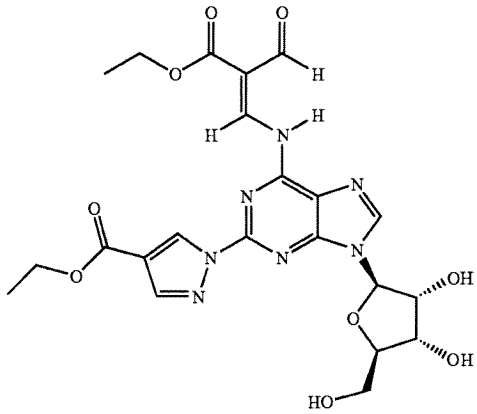
Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting(Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

Purification of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate

A solution of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (100 g) in dimethylsulfoxide (300 ml) was filtered through a 0.6 to 0.8 micron prefilter and a 0.2 micron filter to remove any solid impurities. The filtrate was then slowly added over a period of 1 hour to deionized water (1 liter) with stirring, and the slurry thus produced stirred for not less than 1 hour. The solid was filtered off, washed with deionized water (2×1 liter), and dried under vacuum for not less than 1 hour. The dried product was then slurried again with deionized water (1.5 liter) for not less than 2 hours, filtered off, and washed with deionized water (1 liter) followed by absolute ethanol (750 ml). The purified product was dried under vacuum at a temperature of not more than 40° C. for not less than 12 hours, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate free of any 2-hydrazinoadenosine impurity.

EXAMPLE 5

Preparation of Ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate



A mixture of 2-hydrazinoadenosine (100 g, 0.34 mol), ethyl 2-formyl-3-oxopropionate (242 g, 1.7 mol) and absolute ethanol were charged to a reactor, and the mixture heated to reflux for 2 hours. When the reaction was judged complete, the heat source was removed and the mixture gradually cooled to 5-10° C. over a period of 3 hours. The slurry was stirred for 30 minutes at this temperature, and the mixture filtered. The solid material was washed with cold (5-10° C.) absolute ethanol, and then dried under vacuum at a temperature that did not exceed 40° C., to provide ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)-pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

An elemental analysis gave the following results: C, 48.75%; H, 4.86%; N, 18.05%; O, 27.57. Theoretical: C, 49.72%; H, 4.74%; N, 18.45%; O, 27.09. The analysis corresponds within experimental error limits to the hemihydrate of the desired product. (C, 48.89%; H, 4.81%; N, 18.1%; O, 28.12)

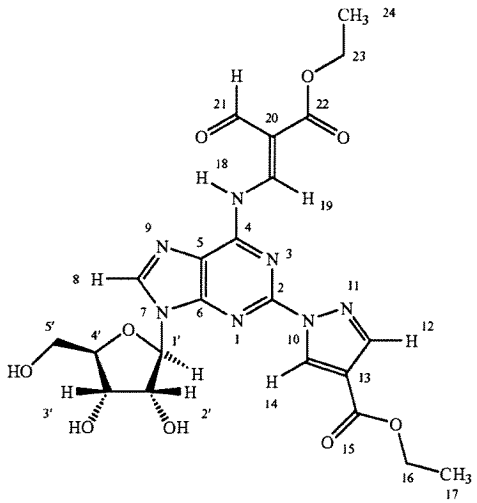
^{1}H and ^{13}C NMR spectra were obtained in the following manner. 20.2 mg of the compound of formula (4) was dissolved in ~0.75 ml of DMSO-d6, and the spectra obtained at ambient temperature on a JEOL ECX-400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were referenced to the DMSO solvent, 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C.

Results

The ^{1}H and ^{13}C chemical shifts are listed in Table 1. Two isomers in a ratio of ~60/30 were observed in both the ^{1}H and the ^{13}C spectra, labeled as major and minor in the table.

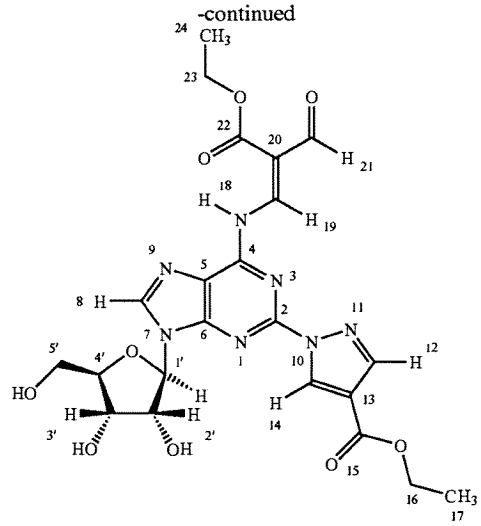
Atom[a]	^{13}C Chemical Shift (ppm)	^{1}H Chemical Shift (ppm)	Multiplicity[b], Splitting (Hz)
21(major)	192.4	9.96	d, 3.6
21(minor)	187.6	9.83	S
22(minor)	167.1	—	—
22(major)	165.2	—	—
15(minor)	161.8	—	—
15(major)	161.7	—	—
6(major)	153.1	—	—
6(minor)	152.9	—	—
2(minor)	149.4	—	—
2(major)	149.3	—	—
19(minor)	148.0	9.22	d, 13.0
4(minor)	147.9	—	—
4(major)	147.8	—	—
19(major)	147.5	9.26	d, 12.4, d, 3.6
8(major)	144.9	8.87	s
8(minor)	144.7	8.85	s
12	143.1	8.20-8.23	m
14(minor)	132.8	9.20	d, ~0.7
14(major)	132.6	9.12	d, ~0.7
5(major)	120.7	—	—
5(minor)	120.6	—	—
13	116.7	—	—
20(minor)	107.2	—	—
20(major)	106.1	—	—
1'(major)	87.9	6.07	d, 5.3
1'(minor)	87.9	6.06	d, 5.3
4'	85.8	4.02	q, 3.9
2'(minor)	74.1	4.62	q, ~5.4
2'(major)	74.1	4.61	q, ~5.4
3'	70.1	4.22	q, 4.2
5'	61.0	3.62, 3.73	m
23, 16	60.3-60.8	4.25-4.39	m
17, 24	14.1-14.2	1.28-1.38	m
18(major)	—	12.51	d, 12.4
18(minor)	—	11.47	d, 13.0
2'-OH(major)	—	5.63	d, 6.1
2'-OH(minor)	—	5.62	d, 6.1
3'-OH	—	5.30	d, 5.1
5'-OH	—	5.08	t, 5.5

The compound of formula (4) was confirmed to be a mixture of the following two isomers:



Major

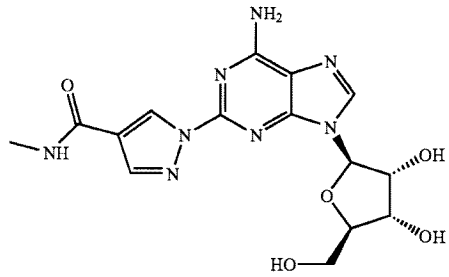
-continued



Minor

EXAMPLE 6

Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from Compound (4)



Aqueous 40% methylamine solution (1300 ml) was placed in a pressure reactor, cooled to 0-5° C., and the product of Example 5 (ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate (100 g) added. The mixture was stirred at 0-5° C. for at least 8 hours, monitoring the reaction for completion. When complete, the mixture was warmed, maintaining the temperature between 50 and 60° C. for 1 hour, and then cooled to less than 30° C. over a period of 1 hour. When the temperature was below 30° C., the mixture was degassed using a pressure of 100-150 mm Hg, allowing the temperature to decrease to 0-5° C. The mixture was stirred at 0-5° C. for at least 1 hour, maintaining the pressure at 100-150 mm Hg. The vacuum was then discontinued and replaced by nitrogen, maintaining the temperature at 0-5° C. for not less than 30 minutes. The solid product was then filtered off, washed with water (3×500 ml), then with absolute ethanol (625 ml). The product was dried under vacuum, not allowing the temperature to exceed 40° C., to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate.

^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6-DMSO-5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting(Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

An elemental analysis gave the following results: C, 43.96%; H, 4.94%; N, 27.94. Theoretical: C, 44.12%; H, 4.94%; N, 27.44%; O, 27.09. The analysis corresponds within experimental error limits to the monohydrate.

We claim:

1. A monohydrate of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, which monohydrate is in a crystalline form.

2. The monohydrate of claim **1**, wherein the crystalline form has a X-ray diffraction pattern as shown in FIG. **3**.

3. The monohydrate of claim **1**, wherein the crystalline form has a thermogravimetric analysis pattern and a differential scanning calorimetry pattern as shown in FIG. **2**.

4. The monohydrate of claim **1**, wherein the crystalline form is obtainable by a method comprising crystallizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in an aqueous protic solvent or an aqueous polar solvent.

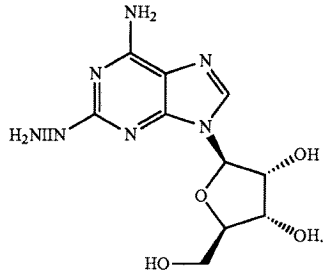
5. The monohydrate of claim **1**, wherein the crystalline form is obtainable by a method comprising crystallizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in a solvent selected from a mixture of ethanol and water and a mixture of dimethylsulfoxide and water.

6. A method for preparing the monohydrate of claim **1**, comprising crystallizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-methylcarboxamide in an aqueous protic solvent or an aqueous polar solvent.

7. The method of claim **6**, wherein the aqueous protic solvent or the aqueous polar solvent is selected from a mixture of ethanol and water and a mixture of dimethylsulfoxide and water.

8. The monohydrate of claim **1**, wherein the crystalline form has a ^{1}H NMR spectrum as shown in FIG. **1**.

9. The monohydrate of claim **1**, wherein the crystalline form is free of any impurity represented by the following structure:



* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,106,183 B2
APPLICATION NO. : 12/765623
DATED : January 31, 2012
INVENTOR(S) : Zablocki et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page, Item (75) Inventor is corrected to read:

-- Jeff Zablocki, Mountain View, CA (US); Elfaith Elzein, Fremont, CA (US); Robert Seemayer, Belmont, CA (US); Travis Lemons, Los Altos Hills, CA (US). --.

Signed and Sealed this
Third Day of March, 2015

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

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This certificate supersedes the Certificate of Correction issued March 3, 2015.

Signed and Sealed this
Twenty-fourth Day of November, 2015

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office

Exhibit B

US009085601B2

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

(10) **Patent No.: US 9,085,601 B2**
(45) **Date of Patent: *Jul. 21, 2015**

(54) **PROCESS FOR PREPARING AN A2A-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS**

(71) Applicant: **Gilead Sciences, Inc.**, Foster City, CA (US)

(72) Inventors: **Jeff Zablocki**, Mountain View, CA (US); **Elfatih Elzein**, Fremont, CA (US); **Robert Seemayer**, Belmont, CA (US); **Travis Lemons**, Los Gatos, CA (US)

(73) Assignee: **Gilead Sciences, Inc.**, Foster City, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/970,372**

(22) Filed: **Aug. 19, 2013**

(65) **Prior Publication Data**

US 2014/0213539 A1 Jul. 31, 2014

Related U.S. Application Data

(63) Continuation of application No. 13/333,872, filed on Dec. 21, 2011, now Pat. No. 8,524,883, which is a continuation of application No. 12/765,623, filed on Apr. 22, 2010, now Pat. No. 8,106,183, which is a continuation of application No. 11/701,699, filed on Feb. 2, 2007, now Pat. No. 7,732,595.

(60) Provisional application No. 60/801,857, filed on May 18, 2006, provisional application No. 60/765,114, filed on Feb. 3, 2006.

(51) **Int. Cl.**

A01N 43/04	(2006.01)
A61K 31/70	(2006.01)
C07H 19/00	(2006.01)
C07H 19/167	(2006.01)
C07H 19/173	(2006.01)
C07H 19/16	(2006.01)

(52) **U.S. Cl.**

CPC ***C07H 19/16*** (2013.01); ***C07H 19/167*** (2013.01)

(58) **Field of Classification Search**

CPC C07H 19/16; C07H 19/167

See application file for complete search history.

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(57) **ABSTRACT**

Disclosed is a synthesis suitable for large scale manufacture of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

5 Claims, 5 Drawing Sheets

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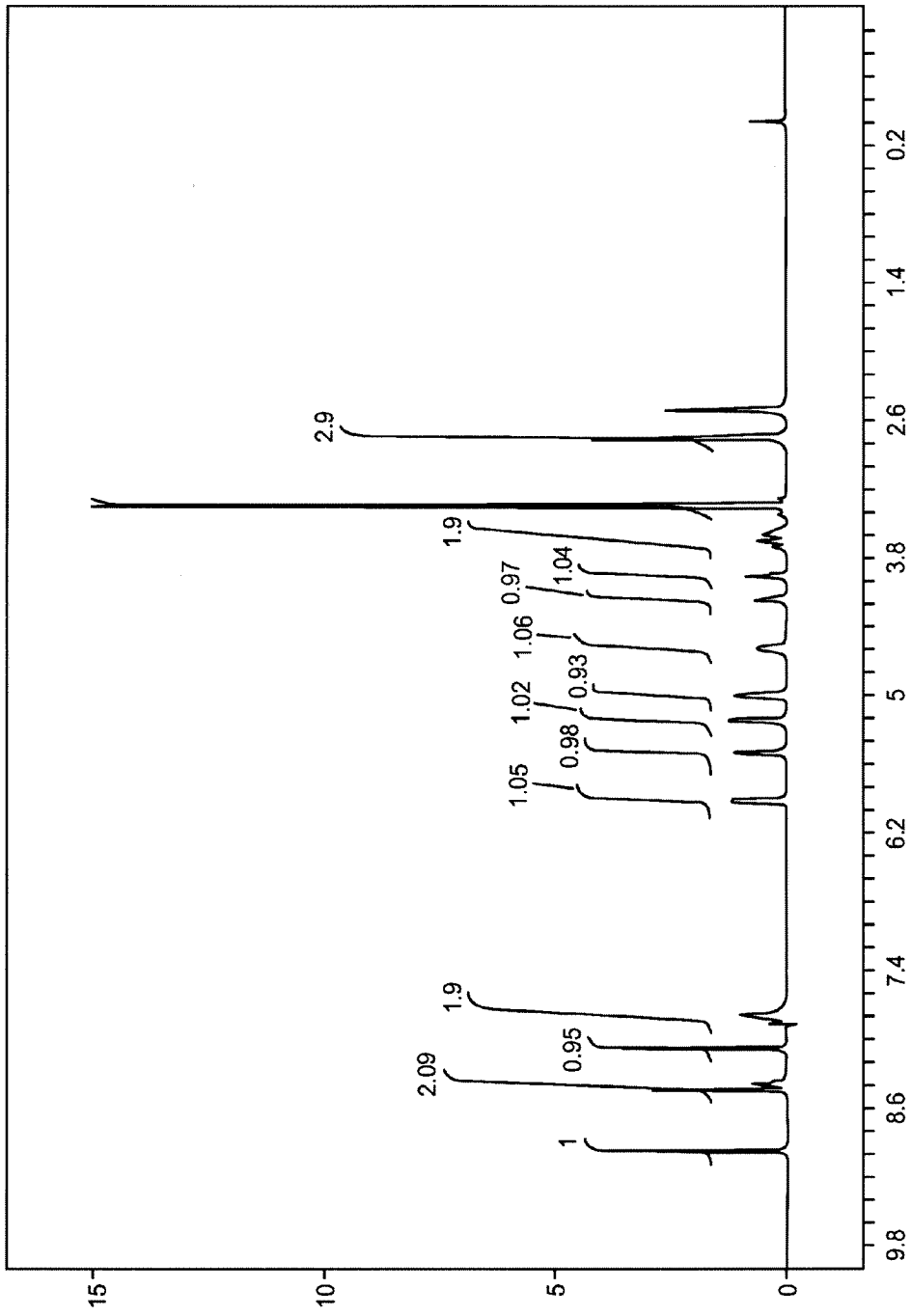

15
10
5
0
9.8
8.6
7.4
6.2
5
3.8
2.6
1.4
0.2
Parts per Million
2.9
1.9
0.97
1.04
1.06
0.93
1.02
0.98
1.05
1.9
0.95
2.09
1

FIG. 1

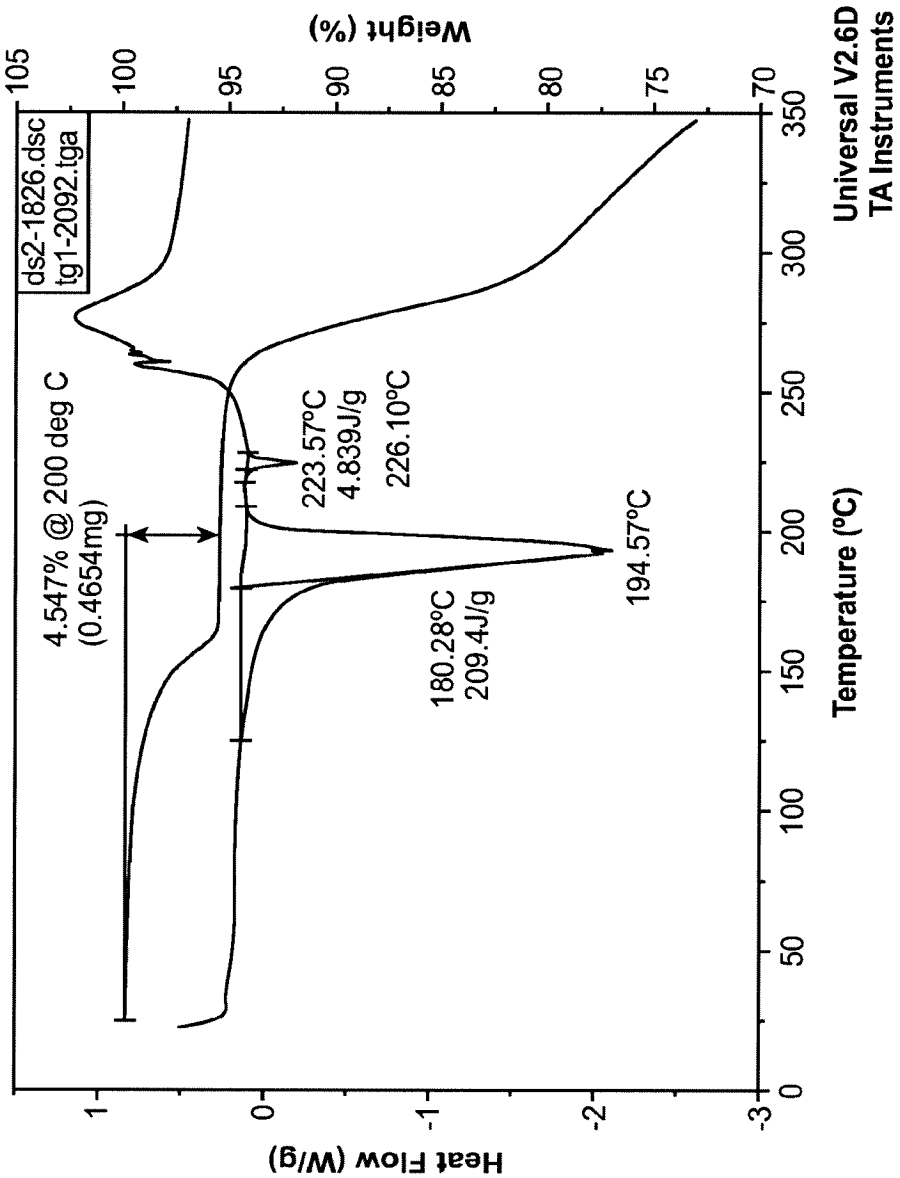

ds2-1826.dsc
tg1-2092.tga
4.547% @ 200 deg C
(0.4654mg)
223.57°C
4.839J/g
226.10°C
194.57°C
180.28°C
209.4J/g
Universal V2.6D
TA Instruments
Temperature (°C)
Heat Flow (W/g)
Weight (%)

FIG. 2

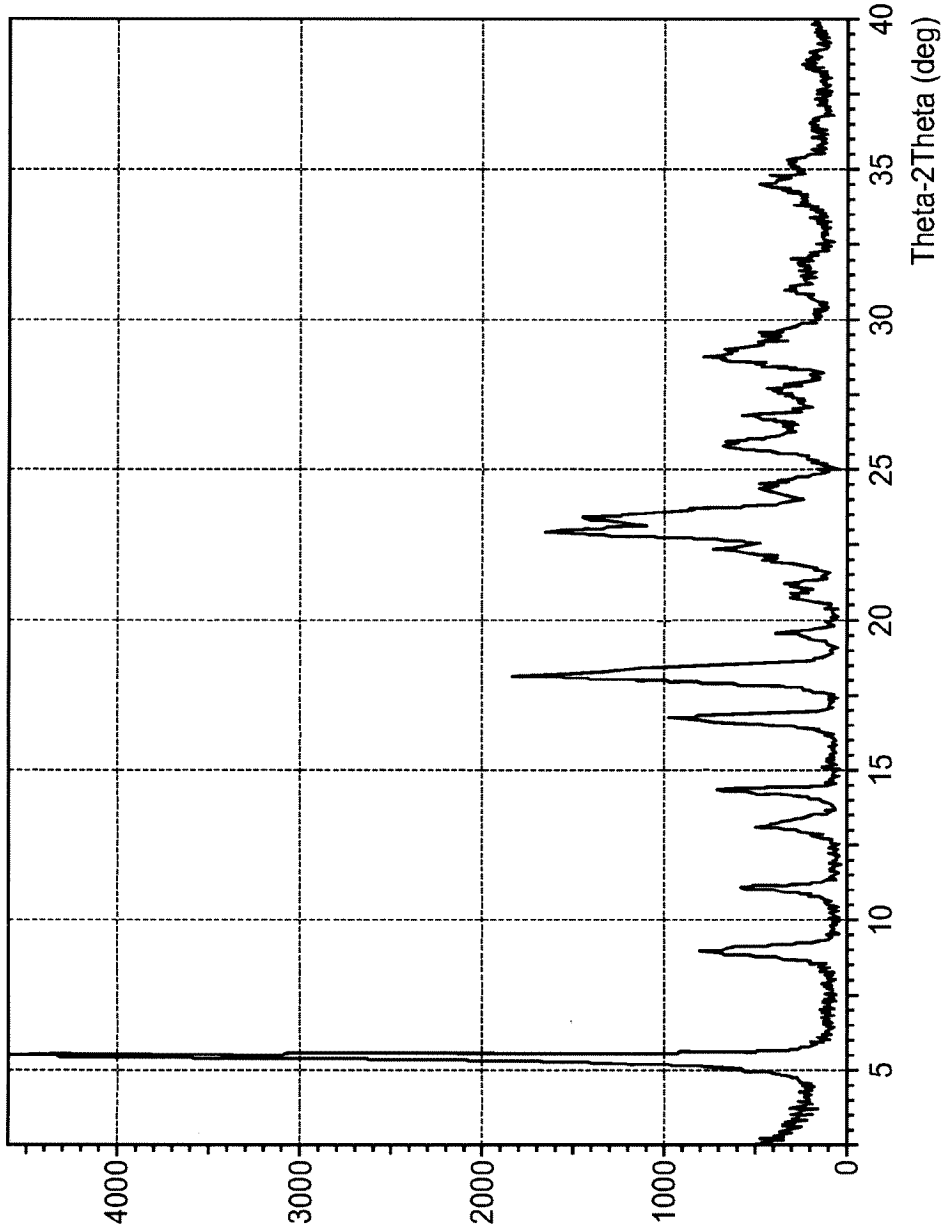


FIG. 3

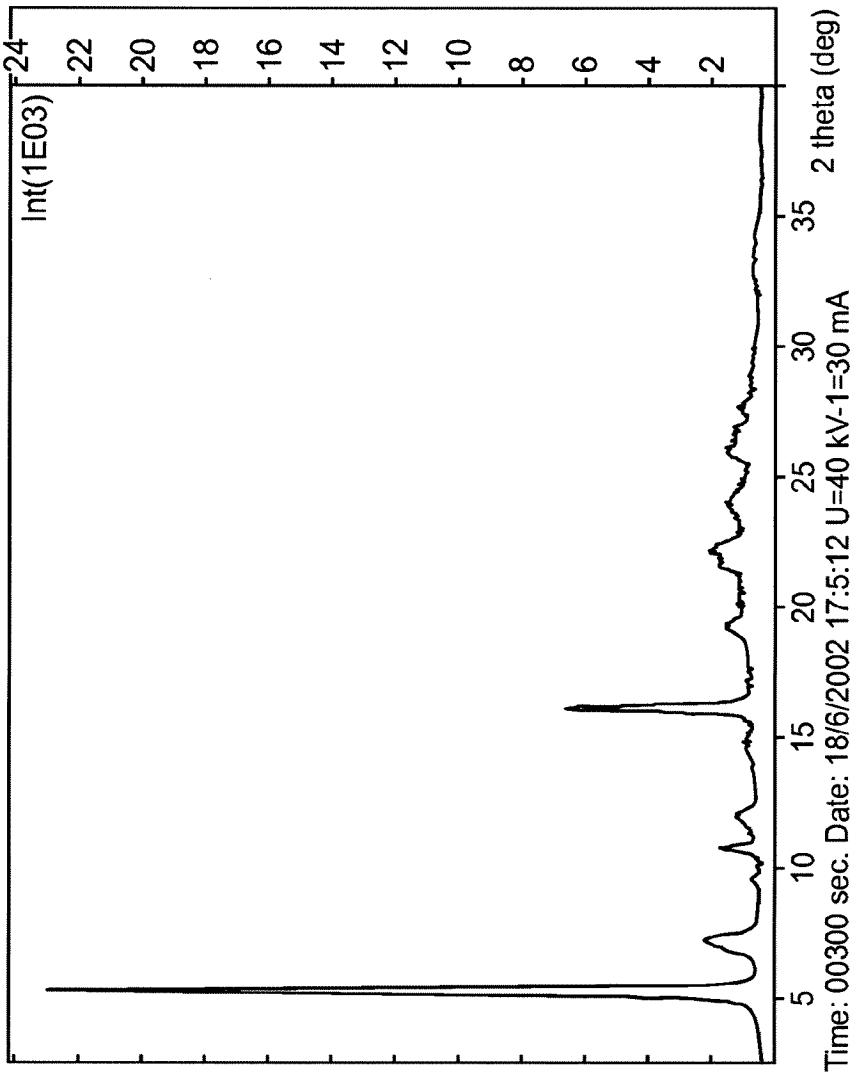

Int(1E03)
24
22
20
18
16
14
12
10
8
6
4
2
5
10
15
20
25
30
35
2 theta (deg)
Time: 00300 sec. Date: 18/6/2002 17:5:12 U=40 kV-1=30 mA

FIG. 4

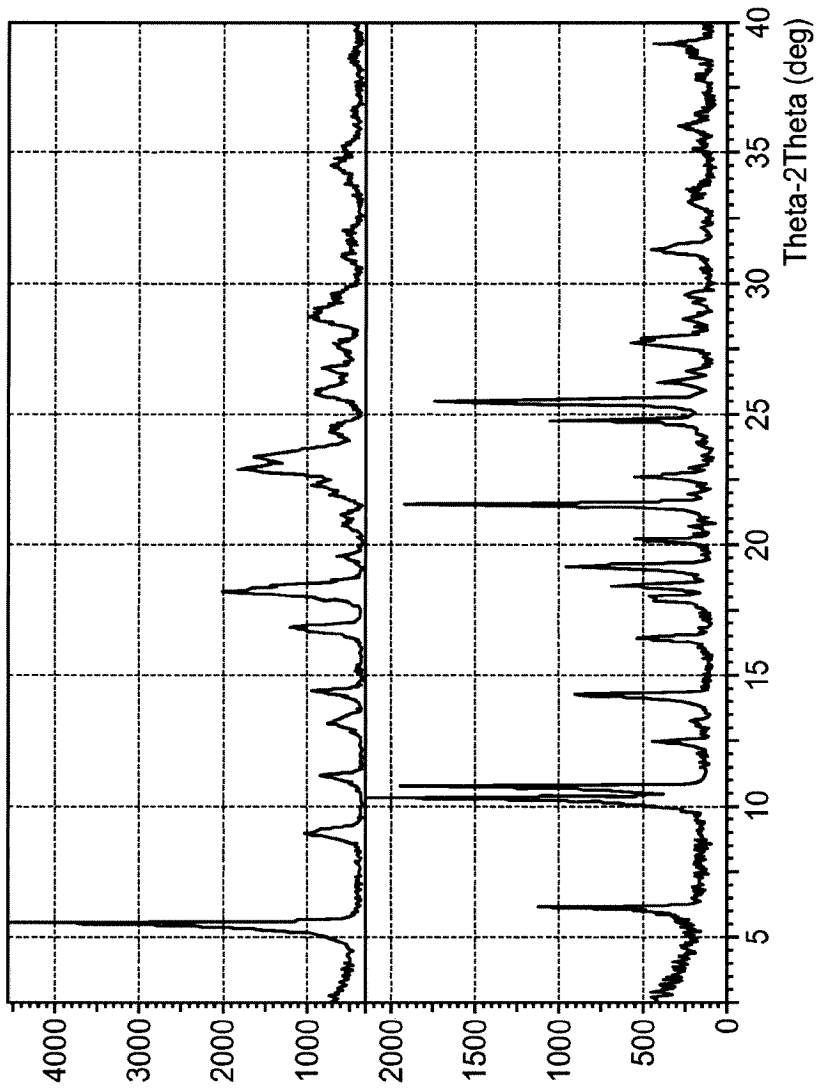

4000
3000
2000
1000
2000
1500
1000
500
0
5
10
15
20
25
30
35
40
Theta-2Theta (deg)

FIG. 5

PROCESS FOR PREPARING AN A2A-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/333,872, filed Dec. 21, 2011, now U.S. Pat. No. 8,524,883, issued on Sep. 3, 2013, which is a continuation of U.S. patent application Ser. No. 12/765,623, filed Apr. 22, 2010, now U.S. Pat. No. 8,106,183, issued on Jan. 31, 2012, which is a continuation of U.S. patent application Ser. No. 11/701,699, filed Feb. 2, 2007, now U.S. Pat. No. 7,732,595, issued on Jun. 8, 2010, which claims priority to U.S. Provisional Patent Application No. 60/801,857, filed May 18, 2006, and to U.S. Provisional Patent Application No. 60/765,114, filed Feb. 3, 2006, which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to a process for the large scale preparation of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

BACKGROUND

Adenosine is a naturally occurring nucleoside, which exerts its biological effects by interacting with a family of adenosine receptors known as A_1, A_{2A}, A_{2B}, and A_3, all of which modulate important physiological processes. One of the biological effects of adenosine is to act as a coronary vasodilator; this result being produced by interaction with the A_{2A} adenosine receptor. This effect of adenosine has been found to be useful as an aid to imaging of the heart, where coronary arteries are dilated prior to administration of an imaging agent (for example thallium 201), and thus, by observation of the images thus produced, the presence or absence of coronary artery disease can be determined. The advantage of such a technique is that it avoids the more traditional method of inducing coronary vasodilation by exercise on a treadmill, which is clearly undesirable for a patient that has a coronary disease.

However, administration of adenosine has several disadvantages. Adenosine has a very short half life in humans (less than 10 seconds), and also has all of the effects associated with A_1, A_{2A}, A_{2B}, and A_3 receptor agonism. Thus the use of a selective A_{2A} adenosine receptor agonist would provide a superior method of producing coronary vasodilation, particularly one with a longer half life and few or no side effects.

A class of compounds possessing these desirable properties was disclosed in U.S. Pat. No. 6,403,567, the complete disclosure of which is hereby incorporated by reference. In particular, one compound disclosed in this patent, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, has been shown to be a highly selective A_{2A}-adenosine receptor agonist, and is presently undergoing clinical trials as a coronary vasodilator useful in cardiac imaging.

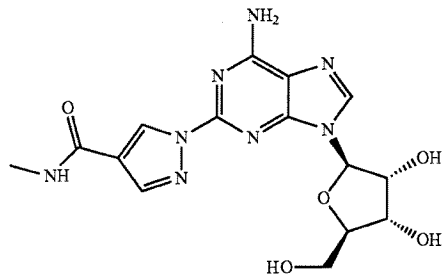
Given the heightened interest in this and similar compounds, it has become desirable to find new methods of synthesis that provide a convenient method for making large quantities of the material in good yield and high purity. The patent that discloses the compound of interest (U.S. Pat. No. 6,403,567) provides several methods for preparing the compound. However, although these methods are suited to small scale syntheses, all synthetic methods disclosed in the patent utilize protecting groups, which is undesirable for large scale syntheses.

Additionally, it was discovered that the desired product (that is (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide) is capable of existing in at least three different crystalline forms, the most stable of which is a monohydrate. This polymorph is stable under relative humidity stress conditions, up to its melting point. Accordingly, it is desirable that the final product produced in the new syntheses is obtained as the stable monohydrate.

SUMMARY OF THE INVENTION

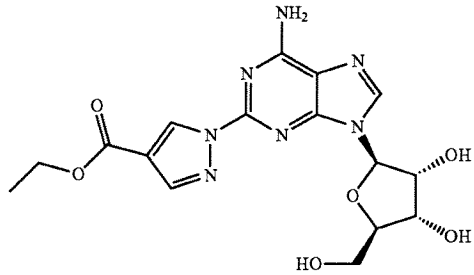
Thus, it is an object of this invention to provide convenient syntheses for the large scale preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, and polymorphs thereof, preferably as its monohydrate. Accordingly, in a first aspect, the invention relates to the preparation of a compound of the Formula I:

Formula I



comprising:
contacting a compound of the formula (3):

(3)



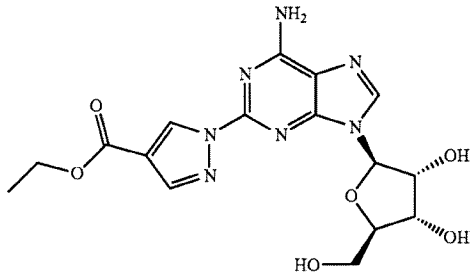
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature of about 0-5° C., followed by warming to about 50-70° C. Alternatively, the reaction is conducted as above but in a sealed pressure reactor.

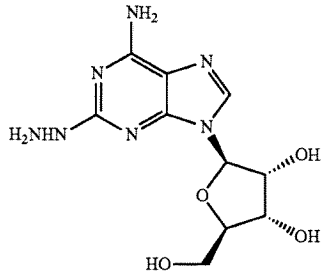
In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

In a second aspect, the invention relates to the preparation of a compound of the formula (3):

(3)



comprising:
contacting a compound of the formula (2):

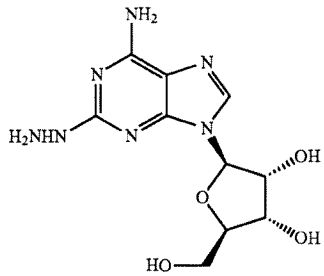


with ethyl 2-formyl-3-oxopropionate.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C., with about 1.1 molar equivalents of ethyl 2-formyl-3-oxopropionate.

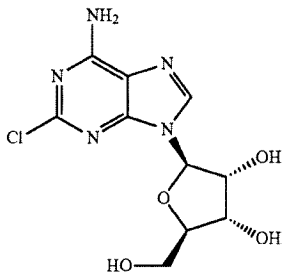
In a third aspect, the invention relates to the preparation of a compound of the formula (2):

(2)



comprising:
contacting a compound of the formula (1):

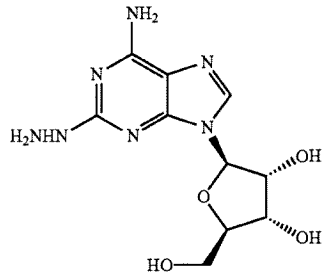
(1)



with hydrazine.

The above described synthesis is suitable for the large scale synthesis of the desired product, which is provided in good yield, although one minor impurity is seen in the final product. This impurity has been shown to be unchanged intermediate of the formula (2); that is, the compound of the formula:

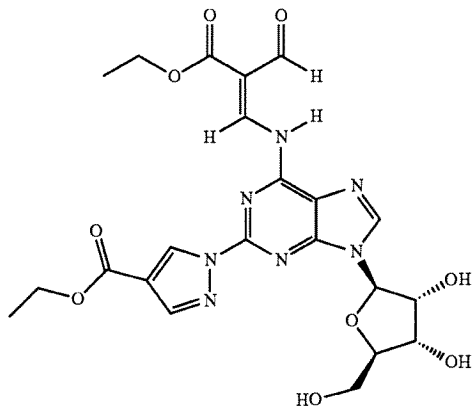
(2)



Although this impurity can be removed from the final product by crystallization, it was decided to seek an alternative synthesis that had all of the advantages of the above synthesis but did not give the compound of formula (2) as an impurity in the final product.

Thus, in a fourth aspect, the invention relates to a method of synthesizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide by contacting a compound of the formula (4):

(4)



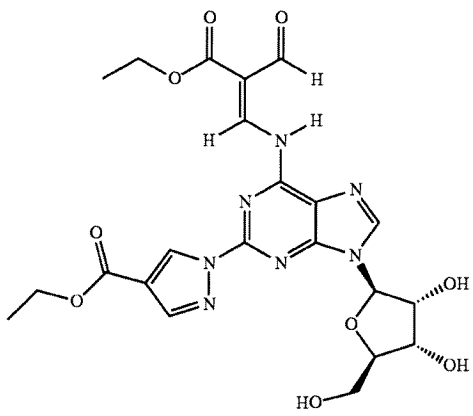
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature of about 0-5° C., followed by warming to about 50-70° C. Preferably, the reaction is conducted in a sealed pressure reactor.

In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

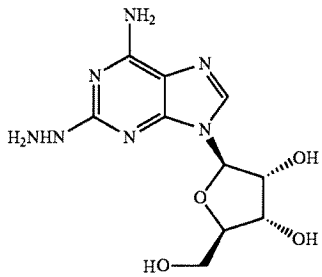
In a fifth aspect, the invention relates to a method of synthesizing a compound of the formula (4):

(4)



comprising contacting a compound of the formula (2):

(2)



with an excess of ethyl 2-formyl-3-oxopropionate, preferably about a 2-10 fold excess, more preferably about a 5-10 fold excess.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C. The ethyl 2-formyl-3-oxopropionate is present in a 5-10 fold excess.

DEFINITIONS AND GENERAL PARAMETERS

FIG. **1** is a ^{1}H NMR spectrum of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (Form A).

FIG. **2** shows the thermal analysis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **3** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **4** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form B.

FIG. **5** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form C as compared to Form A.

As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not.

The term "therapeutically effective amount" refers to that amount of a compound of Formula I that is sufficient to effect treatment, as defined below, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "polymorph" is intended to include amorphous and solvates of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide.

It has been discovered that this compound is capable of existing in at least three different crystalline forms, referred to herein as Form A, Form B, Form C, and an amorphous product.

Form A:

This polymorph can be produced by crystallizing 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from protic solvents, for example ethanol or ethanol/water mixtures, or from a polar solvent, for example dimethylsulfoxide/water. Form A has been shown to be a monohydrate, and is the most stable of the various polymorphs at ambient temperatures. It is stable under relative humidity stress conditions up to its melting point.

Form B:

This polymorph is produced by evaporating under vacuum a solution of 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in trifluoroethanol at ambient temperatures. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. **4**), but it was difficult to determine its constitution, as the X-ray analysis gave disordered broad peaks, and the polymorph contained varying amounts of water. It was found to be difficult to reliably reproduce the preparation of this polymorph.

Form C:

This polymorph is produced by slurrying 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in

acetonitrile for a long period of time at 60° C. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. 5). Polymorph C was shown to be a variable hydrate, which, at elevated temperatures, desolvates to an unstable form.

Amorphous Material:

This polymorph is produced by heating Form A polymorph at a temperature of up to 200° C. This polymorph is unstable in the presence of atmospheric moisture, forming variable hydrates.

Techniques for Analysis of Forms A, B, C and Amorpheous Material

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu Kα radiation. The instrument was equipped with a fine focus X-ray tube, and the tube voltage and amperage were set to 40 kV and 40 mA respectively. The divergence and scattering slits were set at 1" and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5-40° 2θ was used. A silicon standard was used to check the instrument alignment. Data were collected and analyzed using XRD-6000 v.4.1 software.

X-ray powder diffraction (XRPD) analyses were also performed using an Inel XRG-3000 diffractometer equipped with a CPS (Curved Position Sensitive) detector with a 28 range of 120°. The instrument calibration was performed using a silicon reference standard. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The monochromator slit was set at 5 mm by 80 μm. Samples were placed in an aluminum sample holder with a silicon insert or in glass XRPD-quality capillaries. Each capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. Real time data were collected using Cu-Kα radiation at a resolution of 0.03° 20. Typically, data were collected over a period of 300 seconds. Only the data points within the range of 2.5-40° 20 are displayed in the plotted XRPD patterns.

Thermal Analyses

Thermogravimetric (TG) analyses were carried out on a TA Instruments 2050 or 2950 thermogravimetric analyzer. The calibration standards were nickel and Alumel™. Samples were placed in an aluminum sample pan, inserted into the TG furnace, and accurately weighed. The samples were heated in nitrogen at a rate of 10° C./min to either 300 or 350° C. Unless stated otherwise, samples weights were equilibrated at 25° C. in the TGA furnace prior to analysis.

Differential scanning calorimetry (DSC) analyses were carried out on a TA Instruments differential scanning calorimeter 2920. Accurately weighed samples were placed in either crimped pans or hermetically sealed pans that contained a pinhole to allow for pressure release. Each sample was heated under nitrogen at a rate of 10° C./min to either 300 or 350° C. Indium metal was used as the calibration standard. Temperatures were reported at the transition maxima.

Infrared Spectroscopy

Infrared spectra were acquired on Magna 860® Fourier transform infrared (FT-IR) spectrophotometer (Nicolet Instrument Corp.) equipped with an Ever-Glo mid/far IR source, an extended range potassium bromide beamsplitter, and a deuterated triglycine sulfate (DTGS) detector. Unless stated otherwise, a Spectra-Tech, Inc. diffuse reflectance accessory (the Collector™) was used for sampling. Each spectrum represents 256 co-added scans at a spectral resolution of 4 cm^{-1}. Sample preparation for the compound consisted of placing the sample into a microcup and leveling the material with a frosted glass slide. A background data set was acquired with an alignment mirror in place. The spectra represent a ratio of the sample single-beam data set to the background single beam data set. Wavelength calibration of the instrument was performed using polystyrene.

NMR Spectroscopy

Solution phase ^1H NMR spectra of the were acquired at ambient temperature on a Bruker model AM-250 spectrometer operating at 5.87 T (Larmor frequency: 'H=250 MHz). Time-domain data were acquired using a pulse width of 7.5 ps and an acquisition time of 1.6834 second over a spectral window of 5000 Hz. A total of 16,384 data points were collected. A relaxation delay time of 5 seconds was employed between transients. Each data set typically consisted of 128 coaveraged transients. The spectra were processed utilizing GRAMS132 A1 software, version 6.00. The free induction decay (FID) was zero-filled to four times the number of data points and exponentially multiplied with a line-broadening factor of 0.61 Hz prior to Fourier transformation. The 'H spectra were internally referenced to tetramethylsilane (0 ppm) that was added as an internal standard.

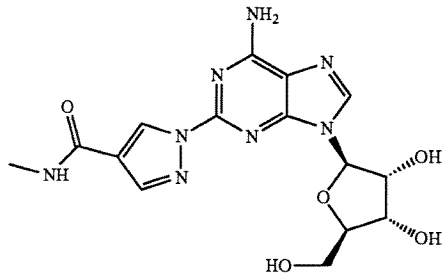
Alternatively, NMR analysis was carried out as described in Example 4.

Moisture Sorption/Desorption Analyses

Moisture sorption/desorption data were collected on a VTI SGA-100 Vapor Sorption Analyzer. Sorption and desorption data were collected over a range of 5% to 95% relative humidity (RK) at 10% RH intervals under a nitrogen purge. Sodium chloride (NaCl) and polyvinyllpyrrolidone (PVP) were used as the calibration standards. Equilibrium criteria used for analysis were less than 0.0100% weight change in 5 minutes, with a maximum equilibration time of 180 minutes if the weight criterion was not met. The plotted data have not been corrected for the initial moisture content.

NOMENCLATURE

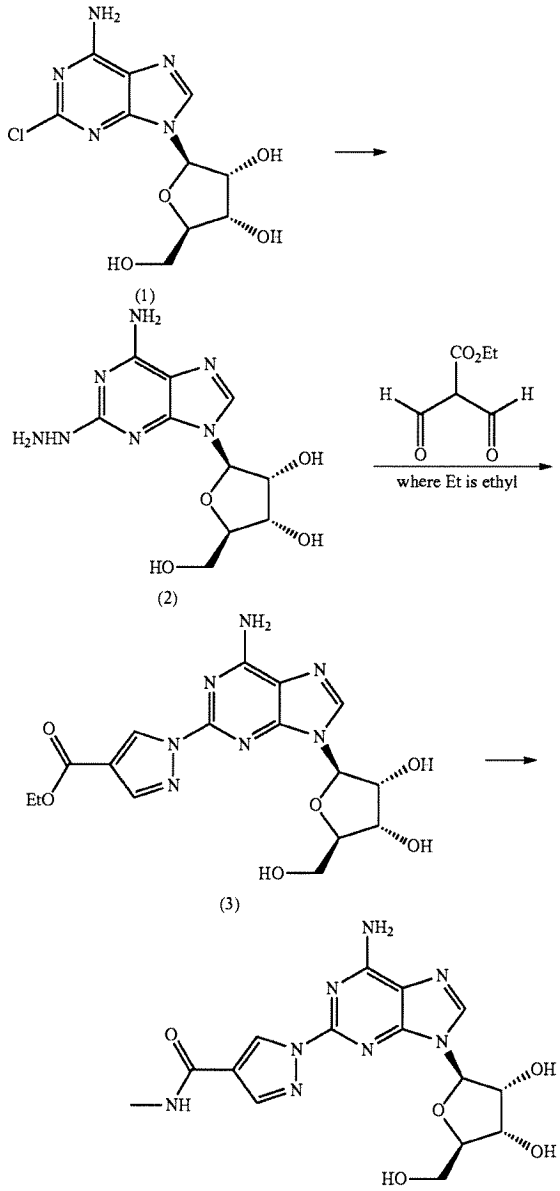
The structure of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is as follows:



Synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide

One method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme I.

REACTION SCHEME I



Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 40° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (3)

The compound of formula (2) is then converted to a compound of formula (3) by reacting with about 1-1.2 molar equivalents of ethyl 2-formyl-3-oxopropionate. The reaction is conducted in a protic solvent, preferably ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure. The product of formula (3) is taken to the next step without purification.

Step 3—Preparation of Final Product

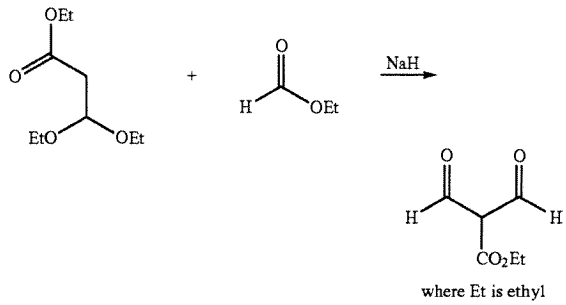
The final product is prepared from the compound of formula (3) by reacting with methylamine, preferably aqueous methylamine. The reaction is carried out at about room temperature, for about 4 hours. The product of Formula I is isolated by conventional means, for example by filtration, washing the solid with cold ethanol, and drying under reduced pressure.

Preparation of Starting Materials

(4S,2R,3R,5R)-2-(6-amino-2-chloropurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol is used as a starting material in step 1. This compound is commercially available.

Ethyl 2-formyl-3-oxopropanoate is used as a starting material in step 2. It is commercially available, or may be made as shown in Reaction Scheme II.

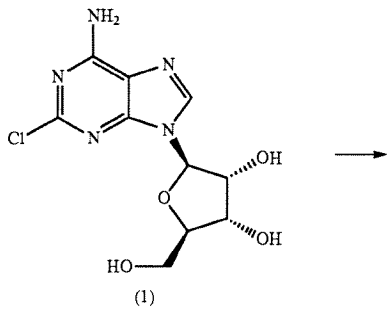
REACTION SCHEME II



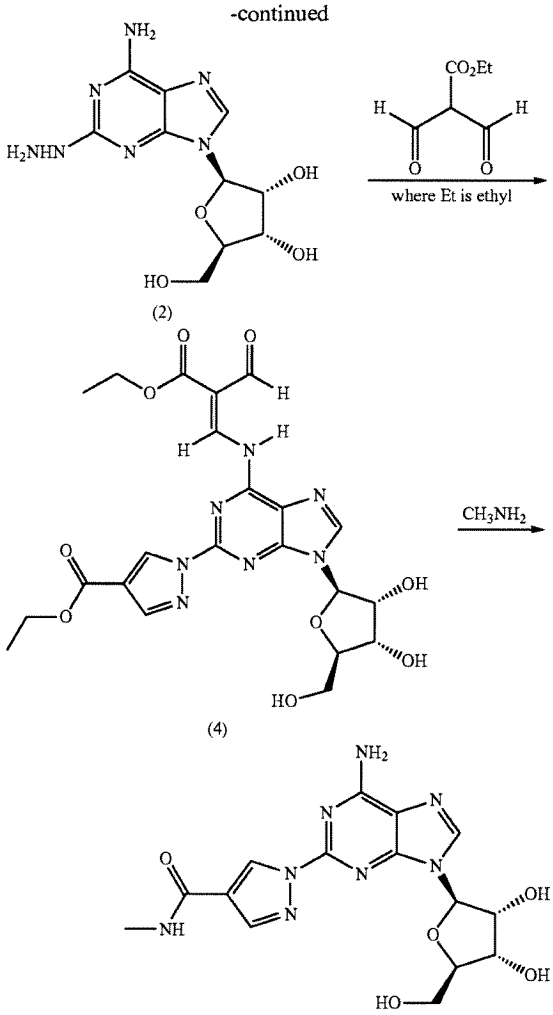
Ethyl 3,3-diethoxypropionate is reacted with ethyl formate in the presence of a strong base, preferably sodium hydride. The reaction is carried out at about 0-5° C., for about 24 hours. The product is isolated by conventional means, for example by the addition of water and extraction of impurities with a conventional solvent, for example t-butylmethyl ether, acidification of the aqueous phase with, for example, hydrochloric acid, followed by extraction with a solvent such as dichloromethane, and removing the solvent from the dried extract under reduced pressure.

A preferred method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme III.

REACTION SCHEME III



-continued



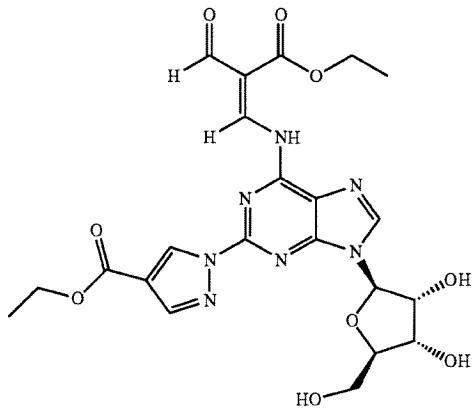
Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 45-55° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by ethanol or isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (4)

The compound of formula (2) is then converted to a compound of formula (4) by reacting with an excess of ethyl 2-formyl-3-oxopropionate, for example a 2-10 fold excess, preferably about 5-10 fold excess. The reaction is conducted in a protic solvent, for example ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure, and the product of formula (4) is taken to the next step without purification.

The compound of formula (4) is drawn as a (2E) alkene derivative, as this is the major isomer formed in this reaction. However, it should be noted that a significant amount of the (2Z) alkene derivative may also be formed in this reaction; that is:



named as ethyl (2Z)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl) pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

Accordingly, although the compound of formula (4) is represented as the (2E) alkene derivative only, the term "compound of formula (4)" is intended to include both the instance where it is solely the (2E) isomer, and the instance where the major portion of the product is the (2E) isomer and a minor portion of the (2Z) isomer is also present. The conversion of the compound of formula (4) to the final product by reaction with methylamine as described in Step 3 proceeds in the same manner whether the compound of formula (4) is present as the (2E) isomer or as a mixture of the (2E) isomer and the (2Z) isomer.

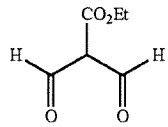
Step 3—Preparation of Final Product

The final product is prepared from the compound of formula (4) by reacting with methylamine, preferably aqueous methylamine. The reaction is initially carried out at about 0-5° C. for about 8 hours, preferably in a pressure reactor, followed by raising the temperature to 50-60° C. over about 1 hour, and maintaining the temperature for 15-30 minutes. The product is isolated by conventional means, for example by cooling to 0-5° C. and maintaining a vacuum for about 1 hour, thus removing the methylamine. The vacuum is removed, and the remaining contents held at 0-5° C. for at least 30 minutes, followed by filtration. The solid thus obtained is washed with water followed by ethanol, and dried under reduced pressure.

This process provides (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate. This polymorph can be further purified by dissolving in dimethylsulfoxide, filtering any solid impurities from the solution, and precipitating the monohydrate from solution by addition of water.

EXAMPLE 1

Preparation of Ethyl-2-formyl-3-oxopropionate

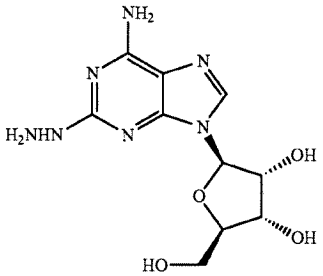


A three- or four-neck round bottom flask equipped with magnetic stir bar, thermocouple, digital thermometer, gas inlet and outlet and addition funnel was flushed with argon. Ethyl 3,3-diethoxypropionate (64.5 g) in tetrahydrofuran were charged to the addition funnel. Sodium hydride (21.2 g of a 60% dispersion) was charged to the reaction flask followed by tetrahydrofuran. The contents of the flask were cooled to 0-5° C. in an ice-bath, and ethyl formate (257 g) was added. The mixture was cooled to 0-5° C. and the contents of the addition funnel added dropwise, maintaining an internal temperature of less than 5° C. The ice-bath was removed and the contents allowed to warm to ambient temperature. Consumption of ethyl 3,3-diethoxypropionate was monitored by TLC analysis. The reaction was quenched by addition of ice-water (10.6 vol), and extracted three times with methyl t-butyl ether (5.4 vol each), and the organic layers discarded. The aqueous phase was acidified with conc. hydrochloric acid to a pH of 1 to 1.5. The acidified aqueous layer was extracted three times with dichloromethane and the combined organic layers dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue distilled under vacuum, to provide ethyl 2-formyl-3-oxopropionate, 27.92 g, 70% yield.

EXAMPLE 2

A. Preparation of 2-Hydrazinoadenosine (2)

(2)



A flask equipped with a mechanical stirrer, gas inlet, gas outlet and thermocouple was flushed with argon. 2-Chloroadenosine hemihydrate (53.1 g) was added, followed by hydrazine monohydrate (134 g). The mixture was stirred while heating to 40-45° C. for 2 hours. The progress of the reaction was followed by TLC analysis. When the reaction was complete, the heat source was removed and ethanol (800 ml) was added. The mixture was stirred for 2 hours at ambient temperature, then the precipitate collected by filtration. The filter cake was washed with ethanol and dried under reduced pressure for 30 minutes. The solids were transferred to a clean flask equipped with a mechanical stirrer and water (300 ml) was added. The suspension was stirred at room temperature for 18 hours, and the solids isolated by filtration. The filter cake was washed with ice-cold water (300 ml) followed by a wash with ice-cold ethanol (300 ml). The solid was dried under reduced pressure to provide 2-hydrazinoadenosine (41.38 g, 81.4% yield, 99.3% purity).

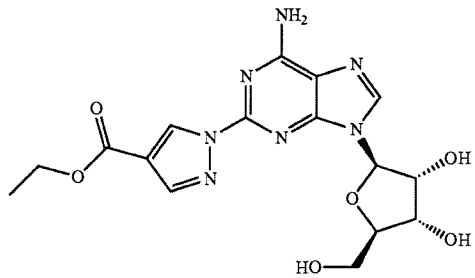
B. Alternative Preparation of 2-Hydrazinoadenosine (2)

A reaction vessel containing hydrazine hydrate (258 g, 250 ml) was heated to 40-50° C. To the warm mixture 2-chloroadenosine hemihydrate (100 g) was added in portions, maintaining the temperature between 45-55° C. The temperature was kept at this temperature for two hours, and then deionized water (500 ml) was added over a period of 30 minutes, maintaining the temperature at 45-55° C. The mixture was then gradually cooled to 0-5° C. over a period of 3 hours, then stirred at this temperature for a further 30 minutes. The solid was then filtered off, and washed with cold (2-5° C.) deionized water (200 ml), followed by ethanol (400 ml). The solid was dried under vacuum for 12 hours, to provide 2-hydrazinoadenosine.

EXAMPLE 3

Preparation of Ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (3)

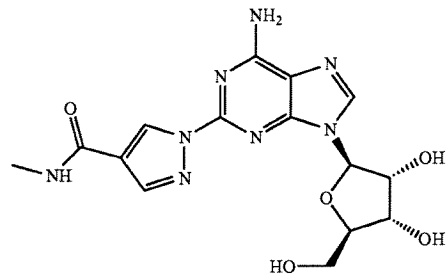
(3)



Ethyl 2-formyl-3-oxopropionate (23.93 g, 0.17 mol) was placed in a flask equipped with mechanical stirrer, gas inlet, gas outlet and reflux condenser. 2-Propanol was added to the flask followed by 2-hydrazinoadenosine (44.45 g, 0.15 mol). The mixture was heated to reflux under stirring for 2-4 hours, following the progress of the reaction by TLC analysis. When the reaction was judged complete, the heat source was removed and the mixture cooled to room temperature. The suspension was cooled under stirring in an ice-bath for 1.5 to 2 hours. The solids were isolated by vacuum filtration, and washed with ice-cold 2-propanol. The product, ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate, was dried under reduced pressure to a constant weight. Yield 54.29 g, purity (by HPLC) 96.6%.

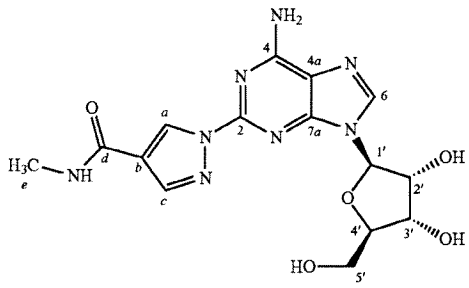
EXAMPLE 4

Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide



A mixture of ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (46.4 g) and methylamine (40% in water, 600 ml) was stirred at ambient temperature for about 4 hours, following the progress of the reaction by HPLC analysis. The majority of the excess methylamine was removed under reduced pressure, and the remaining mixture cooled at 0° C. for 2 hours. The solid material was filtered off, washed with ice-cold 200 proof ethanol, and dried under reduced pressure, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate, 36.6 g, purity 99.6%.

The structure of the material was confirmed by ^{1}H NMR (see FIG. **1** and below). Thermal analysis (see FIG. **2**) provided results consistent with the presence of one molecule of water. X-Ray powder diffraction patterns were obtained (FIG. **3**)



^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6-DMSO—5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting (Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	

-continued

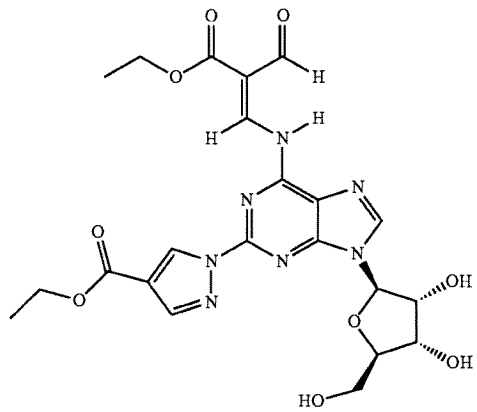
Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting (Hz)
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

Purification of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate

A solution of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (100 g) in dimethylsulfoxide (300 ml) was filtered through a 0.6 to 0.8 micron prefilter and a 0.2 micron filter to remove any solid impurities. The filtrate was then slowly added over a period of 1 hour to deionized water (1 liter) with stirring, and the slurry thus produced stirred for not less than 1 hour. The solid was filtered off, washed with deionized water (2×1 liter), and dried under vacuum for not less than 1 hour. The dried product was then slurried again with deionized water (1.5 liter) for not less than 2 hours, filtered off, and washed with deionized water (1 liter) followed by absolute ethanol (750 ml). The purified product was dried under vacuum at a temperature of not more than 40° C. for not less than 12 hours, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate free of any 2-hydrazinoadenosine impurity.

EXAMPLE 5

Preparation of Ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate



A mixture of 2-hydrazinoadenosine (100 g, 0.34 mol), ethyl 2-formyl-3-oxopropionate (242 g, 1.7 mol) and absolute ethanol were charged to a reactor, and the mixture heated to reflux for 2 hours. When the reaction was judged complete, the heat source was removed and the mixture gradually cooled to 5-10° C. over a period of 3 hours. The slurry was stirred for 30 minutes at this temperature, and the mixture

filtered. The solid material was washed with cold (5-10° C.) absolute ethanol, and then dried under vacuum at a temperature that did not exceed 40° C., to provide ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)-pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

An elemental analysis gave the following results: C, 48.75%; H, 4.86%; N, 18.05%; O, 27.57. Theoretical: C, 49.72%; H, 4.74%; N, 18.45%; O, 27.09. The analysis corresponds within experimental error limits to the hemihydrate of the desired product. (C, 48.89%; H, 4.81%; N, 18.1%; O, 28.12)

^{1}H and ^{13}C NMR spectra were obtained in the following manner. 20.2 mg of the compound of formula (4) was dissolved in ~0.75 ml of DMSO-d6, and the spectra obtained at ambient temperature on a JEOL ECX-400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were referenced to the DMSO solvent, 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C.

Results

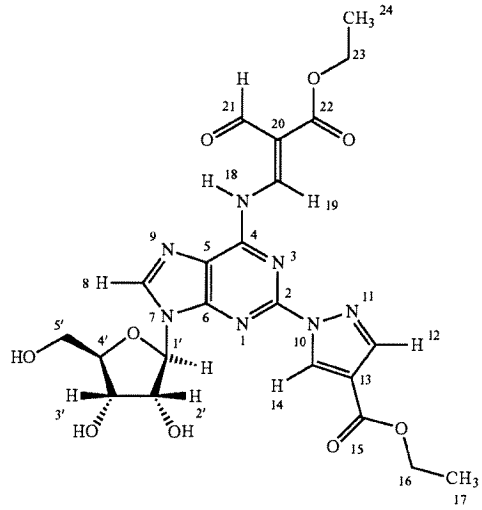
The ^{1}H and ^{13}C chemical shifts are listed in Table 1. Two isomers in a ratio of ~60/30 were observed in both the ^{1}H and the ^{13}C spectra, labeled as major and minor in the table.

Atom[a]	^{13}C Chemical Shift (ppm)	^{1}H Chemical Shift (ppm)	Multiplicity[b], Splitting (Hz)
21(major)	192.4	9.96	d, 3.6
21(minor)	187.6	9.83	S
22(minor)	167.1	—	—
22(major)	165.2	—	—
15(minor)	161.8	—	
15(major)	161.7	—	—
6(major)	153.1	—	—
6(minor)	152.9	—	—
2(minor)	149.4	—	—
2(major)	149.3	—	—
19(minor)	148.0	9.22	d, 13.0
4(minor)	147.9	—	—
4(major)	147.8	—	—
19(major)	147.5	9.26	d, 12.4, d, 3.6
8(major)	144.9	8.87	s
8(minor)	144.7	8.85	s
12	143.1	8.20-8.23	m
14(minor)	132.8	9.20	d, ~0.7
14(major)	132.6	9.12	d, ~0.7
5(major)	120.7	—	—
5(minor)	120.6	—	—
13	116.7	—	—
20(minor)	107.2	—	—
20(major)	106.1	—	—
1' (major)	87.9	6.07	d, 5.3
1' (minor)	87.9	6.06	d, 5.3
4'	85.8	4.02	q, 3.9
2'(minor)	74.1	4.62	q, ~5.4
2'(major)	74.1	4.61	q, ~5.4
3'	70.1	4.22	q, 4.2
5'	61.0	3.62, 3.73	m
23, 16	60.3-60.8	4.25-4.39	m
17, 24	14.1-14.2	1.28-1.38	m
18(major)	—	12.51	d, 12.4
18(minor)	—	11.47	d, 13.0

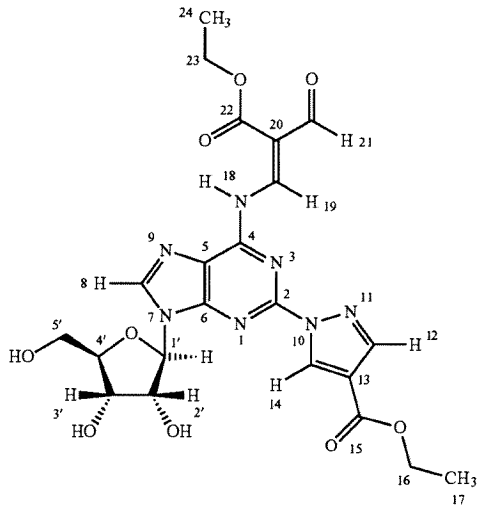
-continued

Atom[a]	^{13}C Chemical Shift (ppm)	^{1}H Chemical Shift (ppm)	Multiplicity[b], Splitting (Hz)
2'-OH (major)	—	5.63	d, 6.1
2'-OH (minor)	—	5.62	d, 6.1
3'-OH	—	5.30	d, 5.1
5'-OH	—	5.08	t, 5.5

The compound of formula (4) was confirmed to be a mixture of the following two isomers:



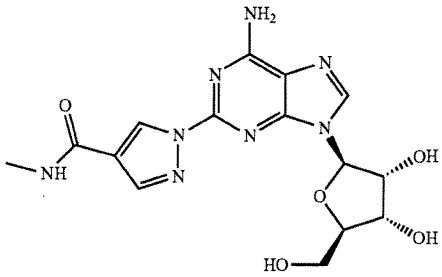
Major



Minor

EXAMPLE 6

Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from Compound (4)



Aqueous 40% methylamine solution (1300 ml) was placed in a pressure reactor, cooled to 0-5° C., and the product of Example 5 (ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate (100 g) added. The mixture was stirred at 0-5° C. for at least 8 hours, monitoring the reaction for completion. When complete, the mixture was warmed, maintaining the temperature between 50 and 60° C. for 1 hour, and then cooled to less than 30° C. over a period of 1 hour. When the temperature was below 30° C., the mixture was degassed using a pressure of 100-150 mm Hg, allowing the temperature to decrease to 0-5° C. The mixture was stirred at 0-5° C. for at least 1 hour, maintaining the pressure at 100-150 mm Hg. The vacuum was then discontinued and replaced by nitrogen, maintaining the temperature at 0-5° C. for not less than 30 minutes. The solid product was then filtered off, washed with water (3×500 ml), then with absolute ethanol (625 ml). The product was dried under vacuum, not allowing the temperature to exceed 40° C., to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate.

^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6-DMSO—5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting (Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

An elemental analysis gave the following results: C, 43.96%; H, 4.94%; N, 27.94. Theoretical: C, 44.12%; H, 4.94%; N, 27.44%; O, 27.09. The analysis corresponds within experimental error limits to the monohydrate.

We claim:

1. A pharmaceutical composition prepared from a crystalline monohydrate form of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide by adding at least one pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim **1**, wherein the monohydrate is substantially free of 2-hydrazinoadenosine.

3. The pharmaceutical composition of claim **2**, wherein the monohydrate is substantially free of other hydrates or amorphous form.

4. The pharmaceutical composition of claim **3**, wherein the monohydrate has a purity of at least about 99.6%.

5. The pharmaceutical composition of claim **4**, wherein the monohydrate exhibits an X-ray powder diffraction pattern having peaks at diffraction angle 2θ (°) of about 6, about 9, about 11, about 13, about 14.5, about 16.5, and about 18 as measured by Cu-Kα1 X-ray powder diffractometry.

* * * * *